

## Advances in Brief

# Potent Antitumor Activity of 2-Methoxyestradiol in Human Pancreatic Cancer Cell Lines<sup>1</sup>

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### Abstract

We examined the effect of 2-methoxyestradiol (2-ME) on the growth and tumorigenesis of human pancreatic cancer cells. 2-ME inhibited the growth of these cell lines (50–90%) in a dose- and time-dependent fashion, and terminal deoxynucleotidyl transferase staining showed that it induced apoptotic cell death. Flow cytometric analysis indicated that 2-ME-sensitive cells showed a prolonged S phase after 48 h of treatment. We used a mouse model for *in vivo* studies of lung metastasis and injected MIA PaCa-2 cells into the tail veins of *nu/nu* mice; lung colonies were formed. Mice given oral 2-ME showed 60% inhibition in the number of lung colonies compared with control, untreated animals. These results suggest that 2-ME may have clinical application for the treatment of pancreatic cancer.

### Introduction

2-ME,<sup>3</sup> a metabolic byproduct of estrogen, is produced by sequential hepatic hydroxylation and methylation from parent compounds (1) and is present in human blood and urine (1, 2). It has been reported recently that 2-ME inhibits endothelial cell proliferation and angiogenesis in an *in vitro* capillary tube

formation model (3). Inhibition of cell growth after 2-ME treatment *in vitro* has been shown due to induction of apoptosis (4).

Apoptosis, or programmed cell death, is an active, gene-directed form of cell death that is different from cell necrosis with respect to its morphology, biochemistry, pharmacology and biological significance. Many types of mammalian cells undergo apoptosis during normal development or in response to a variety of stimuli, including DNA damage, growth factor deprivation, and abnormal expression of oncogene or tumor suppressor genes (5–7). Apoptosis is a widely accepted, important mechanism that contributes to cell growth reduction. 2-ME has been shown to induce apoptosis in a number of different cell types (4, 8, 9).

The present study was undertaken to explore whether 2-ME induces apoptosis in pancreatic cancer cell lines, which exhibit aggressive clinical behavior. We studied the effect of 2-ME on the growth in culture of four pancreatic cancer cell lines and its effect on the metastatic behavior of MIA PaCa-2 in mice. Most of the pancreatic cancer cell lines appeared to be highly sensitive to 2-ME-mediated growth inhibition and underwent apoptotic cell death. Although the molecular mechanism of 2-ME action is not yet clearly understood, it appears to have potential as a therapeutic agent.

### Materials and Methods

**Cell Lines and Tissue Culture.** Four human pancreatic cancer cell lines were used for these studies (PaTu 8902, PaTu 8988t, and PaTu 8988s cell lines were kindly provided by Dr. Hans P. Elsasser, University of Marburg, Berlin, Germany; 10, 11). They were grown in DMEM containing 5% heat-inactivated fetal bovine serum, 5% heat-inactivated horse serum, glutamine, antibiotics, and antimycotics. The cell line MIA PaCa-2 (American Type Culture Collection, Rockville, MD) was grown in high-glucose DMEM supplemented with 10% heat-inactivated fetal bovine serum, glutamine, and antibiotics.

**Cell Proliferation Assay.** Cells were seeded at a density of  $2 \times 10^4$  cells/well in 24-well plates. On the next day, cells were treated with different concentrations of either 2-ME or its inactive analogue 16-epiestriol (ranging from 0.01 to 10  $\mu$ M concentrations). Cells were fed every 2 days; treated cells were replenished with fresh drug. Cells were harvested every other day. Cell growth was monitored first by trypsinizing the cells and then by staining them with crystal violet and counting stained cells using a hemocytometer. All experiments were done in triplicate.

**Cell Cycle Analysis.** Cells were treated with different concentrations of 16-epiestriol (MEC) or 2-ME for 24 h. The concentration of 2-ME that inhibited growth by 50% (IC<sub>50</sub>) in the proliferation assay was determined (1.5  $\mu$ M for MIA PaCa-2, 2  $\mu$ M for PaTu 8988t and PaTu 8902, and 10  $\mu$ M for PaTu 8988s). After washing with PBS, subconfluent cultures were harvested from 100-mm diameter dishes after 24 h of treatment with 2-ME or 16-epiestriol (used as inactive control of

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<sup>3</sup> The abbreviations used are: 2-ME, 2-methoxyestradiol; TdT, terminal deoxynucleotidyl transferase; TUNEL, TdT-mediated dUTP biotin nick-end labeling.

Table 1 Percentage of cells in different cell cycle positions after treatment with 2-ME

	PaTu 8902			PaTu 8988t			MIA PaCa-2			PaTu 8988s		
	Control	MEC <sup>a</sup>	2-ME	Control	MEC	2-ME	Control	MEC	2-ME	Control	MEC	2-ME
G <sub>1</sub>	37.5	37.5	19.8	44.0	40.8	24.7	55.7	55.0	37.3	51.8	53.5	73.9
S	43.5	46.1	57.9	38.5	40.2	69.8	38.3	35.8	49.3	31.4	31.0	15.2
G <sub>2</sub> /M	19.0	16.4	22.3	17.5	19.0	5.5	6.0	9.2	13.4	16.8	15.5	10.9

<sup>a</sup> MEC, 16-epiestriol.

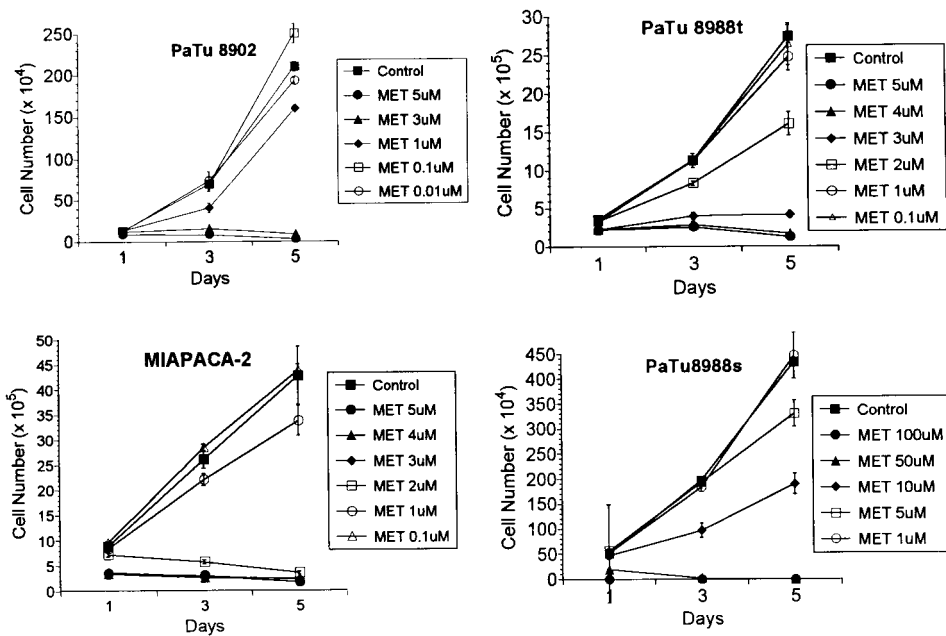


Fig. 1 Proliferation assays of the four pancreatic cancer cell lines used after continuous treatment for 5 days with different doses of 2-ME. Cell numbers relative to those in the control group are shown. An increase of the 2-ME dose from 1 to 2 μM resulted in a 3-to-4-fold increase in growth inhibition in PaTu 8902, PaTu 8988t, and MIA PaCa-2 cells. PaTu 8988s cells were relatively resistant, with only 50% growth inhibition after treatment with 10 μM 2-ME.

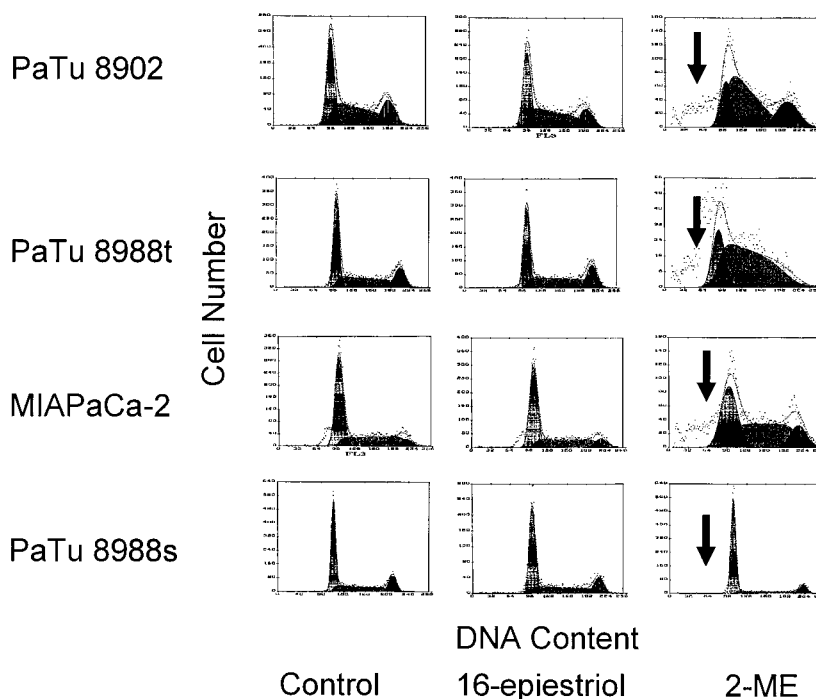
2-ME used in Table 1 and Fig. 3), fixed in 70% ethanol, and stored at 4°C until use. Fixed cells were incubated with 50 μg/ml of propidium iodide and 10 μg/ml of RNase A at 37°C for 30 min. Cell cycle analysis was done using an EPICS Profile II flow cytometer (Coulter Corp., Hialeah, FL). Data were analyzed using the Coulter Cytological program. All experiments were repeated at least two times.

**TUNEL Assay.** A TUNEL assay was performed to detect apoptotic cells *in situ*, as described previously (12). Briefly, cells were seeded in chamber slides (Becton Dickinson, Franklin Lakes, NJ). The cells were set up in chamber slides at 1 × 10<sup>5</sup> cells/chamber. In the treatment group, the cells were incubated with 2-ME (1.5 μM for MIA PaCa-2, 2 μM for PaTu 8902 and PaTu 8988t, and 10 μM for PaTu 8988s). Control and treated slides were then fixed in 1:1 ethanol and acetone for 20 min at -20°C. Next, endogenous peroxidase was blocked with methanol containing 3% H<sub>2</sub>O<sub>2</sub> for 15 min at room temperature. The samples were incubated with 0.1% Triton X-100 and 0.1% sodium citrate at 4°C for 2 min. For positive controls, slides were treated with 0.25 units/L DNase I for 20 min. Control and treated slides were then incubated in TdT buffer [30 mM Tris (pH 7.2), 140 mM sodium cacodylate, and 1 mM cobalt chloride] and covered with 100 units/ml TdT (Life Technologies, Inc., Gaithersburg, MD) and 0.2 mM biotinylated 16-dUTP (Boeh-

ringer Mannheim, Indianapolis, IN) for 2 h at 37°C. The reaction was terminated by incubation in transferring buffer (300 mM sodium chloride and 30 mM sodium citrate) for 30 min at room temperature. After a wash with PBS, the slides were incubated with 2% BSA for 10 min at room temperature. The slides were then incubated with 1:10 peroxidase-conjugated streptavidin (Dako Corp., Carpinteria, CA) for 30 min at room temperature, washed with PBS, and stained with diaminobenzidine for 2 min at room temperature. The cells were counterstained with Harris hematoxylin (Sigma Chemical Co., St. Louis, MO). Brown cells were considered to be apoptotic. Seven fields in every chamber were counted, and the average percentage of brown cells was calculated.

**In Vivo Experiments.** Twenty female athymic mice, 4–6 weeks of age, were used for our studies. To reduce immune function further, animals were irradiated with 350 rads from a <sup>137</sup>Cs source. The next day, 70–80% confluent MIA PaCa-2 cells were trypsinized, washed once in HBSS, and resuspended in PBS. One hundred μl containing 3 × 10<sup>6</sup> MIA PaCa-2 cells were injected into the mice, through the tail veins, to induce lung metastases. The animals were randomly grouped into two groups of 10. 2-ME was dissolved in 2% DMSO of olive oil to obtain a solution containing 1 mg of 2-ME in 50 μl. 2-ME was administered p.o. using an intubation needle. The animals were

**Fig. 2** Cell cycle analysis of cell lines after 24 h of treatment with 2-ME. The concentrations corresponded to the  $IC_{50}$  in the proliferation assays: 2  $\mu M$  for PaTu 8902 and PaTu 8988t, 1.5  $\mu M$  for MIA PaCa-2, and 10  $\mu M$  for PaTu 8988s. No difference was seen between the control and 16-epiestriol-treated groups. After treatment with 2-ME, the population of apoptotic cells increased dramatically in the 2-ME-sensitive cell lines (arrows). In contrast, the 2-ME-resistant PaTu 8988s cell line showed no difference in the cell cycle when compared with the control and 16-epiestriol-treated groups.



fed daily with 50  $\mu l$  of the above-mentioned solution with or without 2-ME. Blinded treatment was performed from day 3 through day 21 after tumor cell injection. After treatment, the mice were killed, their lungs were harvested and fixed with Fekete's solution (60% ethanol, 3% formaldehyde, and 4% glacial acetic acid) after intratracheal injection of a 15% India ink solution. The total number of unstained colonies on the lung surface was counted under a stereoscopic microscope by two investigators without knowledge of the treatment groups.

**Immunohistochemical Staining for Angiogenesis Using a CD31 Antibody.** Two extra groups of five animals each (2-ME treated and nontreated) were used for the immunohistochemical staining. After the mice were fed daily for 3 weeks with 2-ME at the above-mentioned concentrations, the mice were killed, and the lungs were harvested and embedded in OCT compound (Miles, Inc., Elkhart, IN) for frozen sections. After being blocked with 5% goat serum and 1% horse serum, the slides were incubated with rat anti-mouse CD31 (PECAM-1; PharMingen, San Diego, CA) at 4°C overnight. Because primary antibody was rat anti-mouse, a secondary antibody, peroxidase-conjugated anti-rat IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) was used. CD31 was visualized with diaminobenzidine. The number of vessels of a total of 30 lung colonies was counted in each group. The average number of vessels of each group was compared.

## Results

Actively proliferating human pancreatic cancer cell lines were treated with different concentrations of 2-ME, and a cell growth assay was performed. Control, untreated cells displayed exponential growth characteristics over 1–5 days (Fig. 1). The rate of proliferation was significantly slower for these cell lines

after treatment with 2-ME. An initial dose-response study indicated that all cell lines, except one, showed similar kinetics of growth inhibition. The PaTu 8902, PaTu 8988t, and MIA PaCa-2 cell lines showed marked growth inhibition (50–90%) after 5 days of treatment with 2  $\mu M$  2-ME in culture. The  $IC_{50}$  of 2-ME ranged from 2 to 10  $\mu M$  among the cell lines. The PaTu 8988s cell line was less sensitive to 2-ME than the others. After 48 h of 2-ME treatment, the cells were examined under a phase contrast microscope. The cells had shrunken and retracted from the neighboring cells, and the nuclei were fragmented, a characteristic feature of apoptosis. However, no morphological changes were observed when these cell lines were treated with 16-epiestriol, an inactive metabolic byproduct of estrogen with no effect on cell growth (data not shown). These results suggest that 2-ME action on these cell lines was specific, and it inhibited the pancreatic cancer cell growth by inducing apoptosis in a time- and dose-dependent fashion. Moreover, it is important to note that although 2-ME can inhibit the growth of pancreatic cell lines, it had no effect on normal bronchial cells, even when treated with much higher concentrations (4).

2-ME-induced apoptosis of pancreatic cancer cell lines was studied further using flow cytometry and TUNEL assay, as described in "Materials and Methods." When the cell lines were exposed to 2  $\mu M$  2-ME for 24 h, a significant increase in cells with apoptotic morphological changes was observed. After 24 h of treatment, cells were stained with propidium iodide and processed for flow cytometric analysis of the cell cycle and to quantitate the apoptotic cell population (Table 1). Treatment with 2-ME that resulted in growth inhibition elicited a prominent, prolonged accumulation of cells at S phase in the three cell lines that were more sensitive to the treatment. However, in the

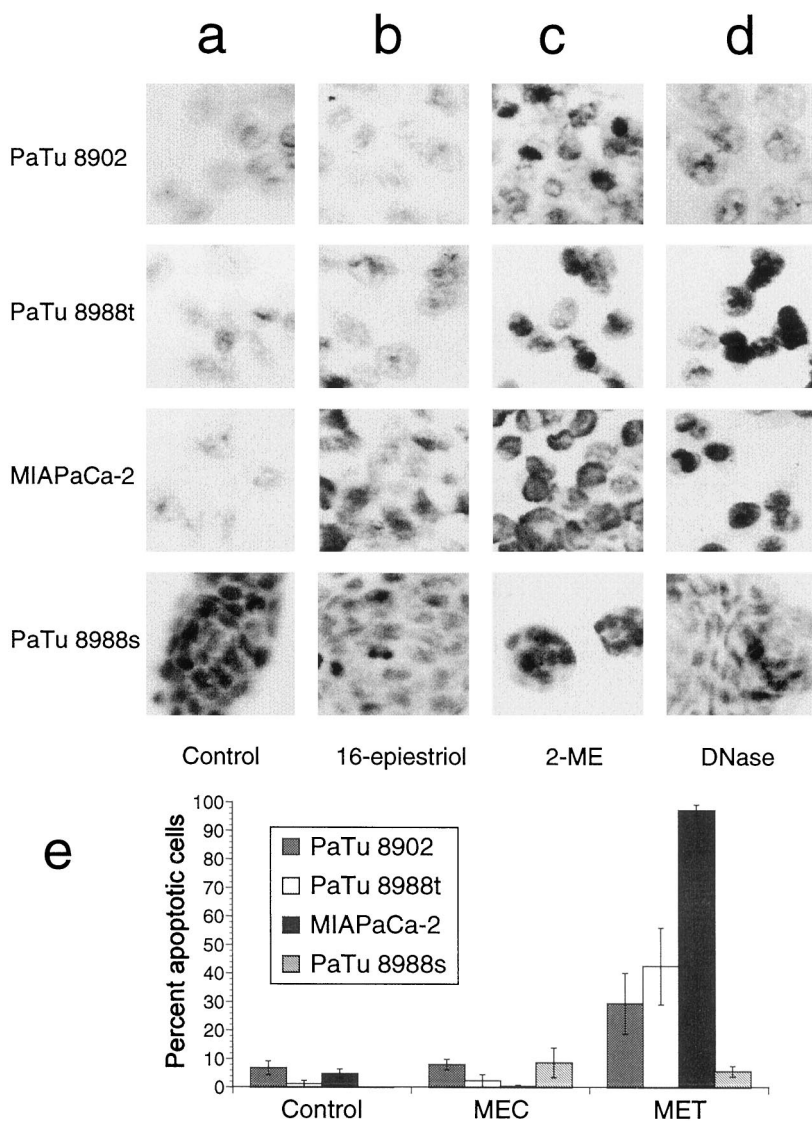


Fig. 3 Apoptotic cell death as revealed after TUNEL staining. Control untreated cells (a) or cells after 24 h of treatment with 16-epiestriol (b), 2-ME (c), or DNase (d). DNase served as a positive control. The concentrations of 2-ME were the same as in Fig. 2. Apoptotic cells appeared dark after staining (c). In contrast, no increased staining was seen in cells treated with 16-epiestriol (b). PaTu 8988s cells showed the same staining intensity with all treatments (a–d). There was a significant increase in the percentage of apoptotic cells after 2-ME treatment (e) in all cell lines except PaTu 8988s. MIA PaCa-2,  $P < 0.0005$ ; PaTu 8902,  $P = 0.0001$ ; PaTu 8988t,  $P = 0.005$ ; PaTu 8988s,  $P =$  not significant. Bars, SE.

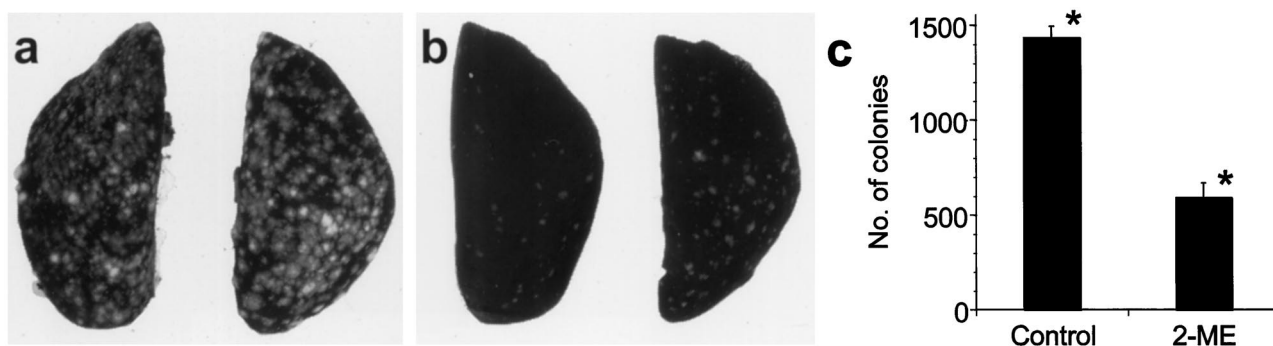
PaTu 8988s cell line, which was relatively less sensitive, no significant effect on the cell cycle was seen.

The 2-ME-induced apoptotic cell population was examined using flow cytometry (Fig. 2). A considerable fraction of the 2-ME-treated cells appeared as sub- $G_1$ , compared with control, untreated cells or 16-epiestriol-treated cells. Thus, the profile of DNA content in the three sensitive cell lines, PaTu 8902, PaTu 8988t, and MIA PaCa2, indicated that a significant fraction had undergone apoptotic cell death after 2-ME treatment. In contrast, the PaTu8988s cell line, which was relatively resistant to 2-ME-mediated growth inhibition, showed little apoptotic cell death on flow cytometric analysis. These results indicate that 2-ME inhibits the growth in the three sensitive pancreatic cancer cell lines by inducing apoptotic cell death.

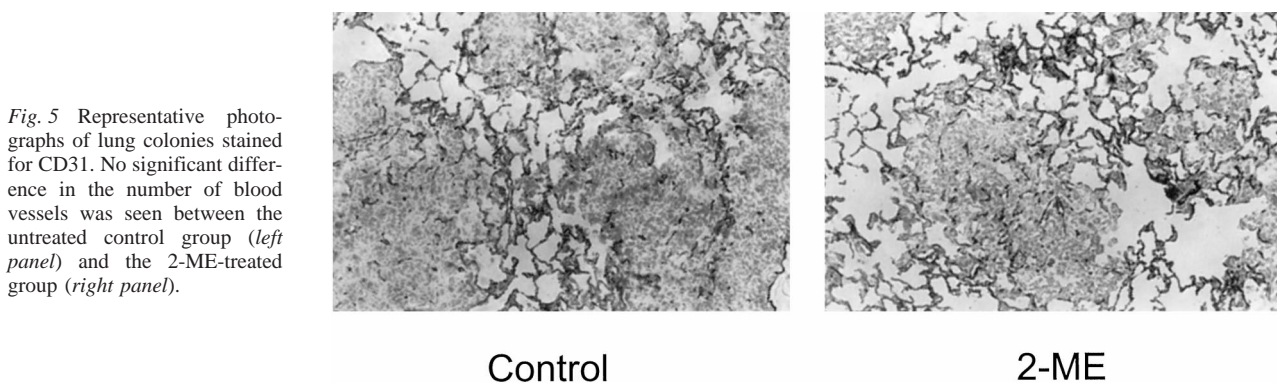
2-ME-induced DNA fragmentation was further examined *in situ* using the TUNEL assay (Fig. 3, a–d). The characteristic TUNEL staining was observed when the PaTu 8902, PaTu8988t, and MIA PaCa-2 cell lines were exposed to  $2 \mu\text{M}$

2-ME for 48 h, and a high percentage of apoptotic cells was detected. However, control, mock-treated cells or 16-epiestriol-treated cells showed little DNA fragmentation, but 2-ME-treated cells displayed many apoptotic bodies. 2-ME treatment resulted in 30–90% apoptotic cell death in these cell lines (~90% in the MIA PaCa-2 cell line).

To study the antitumor effect of 2-ME *in vivo*, we compared the growth and number of metastatic lung colonies in treated and untreated control nude mice 21 days after the MIA PaCa-2 cells were injected into the tail veins (Fig. 4, a and b). There were 10 animals in each group, as described in “Materials and Methods.” Animals were given oral 2-ME, and results showed that 2-ME-treated mice had a 60% lower incidence of lung colonies than control untreated animals (Fig. 5); this result was statistically significant ( $P < 0.0005$  by *t* test analysis). This experiment was repeated three times and showed similar results (Table 2). The animals did not have any signs of toxicity, such as weight loss or modified behavior, during 2-ME treatment. All



**Fig. 4** Representative photomicrographs of lung colonies in untreated control (*a*) and 2-ME-treated (*b*) animals. The figure is one representative experiment of three with similar results. *c*, average number of metastatic colonies on the lung surfaces of the untreated and the treated groups. Bars, SE (\*,  $P < 0.0005$ ).



**Fig. 5** Representative photographs of lung colonies stained for CD31. No significant difference in the number of blood vessels was seen between the untreated control group (*left panel*) and the 2-ME-treated group (*right panel*).

animals injected with MIA PaCa-2 cells developed lung colonies. To examine the effect on angiogenesis in the lung colonies, immunohistochemical staining for CD31, a specific marker for endothelium, was performed. However, no significant difference in the number of blood vessels inside the colonies was seen (Fig. 5).

## Discussion

2-ME appears to have a very distinct effect on pancreatic cell proliferation. Results indicated that 2-ME-mediated inhibition of pancreatic cancer cell growth was due to apoptotic cell death. The effect of 2-ME was concentration dependent, with an  $EC_{50}$  value of 2–5  $\mu\text{M}$ , and under our experimental conditions, spontaneous apoptotic death was very low ( $\leq 1\%$ ). However, a significant number of apoptotic cells were detected 24 h after treatment with 2-ME.

Apoptosis induced by various other agents appears to be mediated by a common set of downstream elements that act as regulators and effectors of apoptotic cell death. In many cases, p53 is required for apoptosis in a number of models, and stabilization of p53 leads cells to undergo apoptosis (4, 13, 14). We have shown previously that in lung cancer cell lines, 2-ME induces endogenous wild-type p53 protein posttranscriptionally with associated bypass of the G<sub>1</sub>-S checkpoint and causes p53-dependent apoptosis (4). Overexpression of p53 in response to several death stimuli induces apoptosis. We, therefore, exam-

ined the p53 gene status in our four pancreatic cancer cell lines. Western blot analysis of the p53 protein using an anti-p53 monoclonal antibody, PCR-single strand conformational polymorphism analysis of exons 4–9, and DNA sequencing indicated that all of these lines harbor mutated p53. PaTu 8988t and PaTu 8988s, both deriving from the same tumor, have a C-to-T mutation in codon 282, whereas PaTu 8902 harbors a T-to-A mutation in codon 176. MIA PaCa-2 cells revealed a C-to-T mutation in codon 248 (data not shown). Therefore, apoptosis induction by 2-ME contrasted with the previous results, indicating that 2-ME elicited p53-independent apoptotic cell death in these pancreatic cancer cell lines.

17 $\beta$ -Estradiol has been reported to have a  $\geq 1000$ -fold higher binding affinity to the cytosolic estrogen receptor than that of 2-ME (15). On the other hand, 2-ME showed much more potent activity for apoptosis induction in the cells compared with 17 $\beta$ -estradiol and 16-epiestriol (2, 4, 8). Moreover, the effect of 2-ME action is independent of estrogen receptor expression because blocking the estrogen receptors by tamoxifen does not abrogate 2-ME-induced apoptosis.<sup>4</sup> These data therefore suggest that 2-ME acts through a separate pathway. Thus, our work and other studies (3) suggest that 2-ME has a pleiotropic effect on

<sup>4</sup> T. Mukhopadhyay and J. A. Roth, unpublished observations.

Table 2 Effect of 2-ME on lung colony formation  
Values are mean  $\pm$  SE.

	Average	$\pm$ SE	No. of animals
Experiment 1			
Control	1155	67	5
2-ME	567 <sup>a</sup>	60.2	5
Experiment 2			
Control	1126	53.7	10
2-ME	404 <sup>a</sup>	76.8	10
Experiment 3			
Control	1431	60.5	10
2-ME	589 <sup>a</sup>	78.7	10

<sup>a</sup> Significantly less than controls.  $P < 0.001$ , by Student's *t* test.

cell growth and apoptosis of cells that depends on the tissue origin and genetic makeup. These results further suggest an association between apoptosis induction and inhibition of cell proliferation by 2-ME and that 2-ME inhibited pancreatic cancer cell proliferation through an apoptotic mechanism.

Angiogenesis, the generation of new capillaries from pre-existing vessels, is critical to cancer progression (16, 17). Angiogenesis requires enzymatic degradation of the basement membrane, then vascular endothelial cell migration into the perivascular space, proliferation and alignment to form tubular structures, and finally, new vessel formation (16, 17). However, the molecular mechanism leading to pathological angiogenesis remains to be elucidated. Tumor angiogenesis has long been thought to be important for the growth of solid tumors (18), and this view is strengthened by the fact that 2-ME inhibits both the neovascularization and growth of s.c. melanoma and sarcoma tumors (2). However, we find no significant difference in vessel density among lung colonies in untreated *versus* 2-ME-treated mice. Nevertheless, this does not completely eliminate antiangiogenesis as a mechanism of growth inhibition of tumor colonies in our model because inhibition of neovascularization could limit the size of the lung colonies.

An earlier report indicated that 2-ME treatment of the estrogen-dependent cell line MCF-7 caused G<sub>2</sub>-M arrest associated with the depolymerization of tubulin (19). However, we observed no such effect of 2-ME on the tubulin structure in immunofluorescence studies of our lung cancer cell lines (data not shown) nor in A431 cells, even at concentrations of 20  $\mu$ M (20). It has been further suggested that 2-ME caused mitotic arrest in a leukemia cell line by inhibiting the calmodulin pathway (20). However, when pancreatic cancer cell lines were treated with 2  $\mu$ M of 2-ME, there is an effect on the distribution of tumor cells in the different phases of the cell cycle (4). A significant proportion of the cells are blocked at S phase. The molecular mechanism that blocks the cells to S phase has yet to be identified. Using Western blot analysis, we examined the E2F1 and Rb gene product, which have been implicated in the progression of the cells to S phase, but noticed no significant difference in the E2F1 expression or in Rb phosphorylation state (data not shown). It is interesting to note that PaTu8988s cells, which were not blocked at S phase after 2  $\mu$ M 2-ME treatment, could avoid the 2-ME-mediated apoptotic cell death. Possibly, conflicting signals that caused premature entry of the cells to S phase and to retain the cells at

S phase for a long time could result in the decision of a cell to undergo programmed cell death.

2-ME appears to have several unique features: (a) it is a nontoxic metabolic byproduct of estrogen present in normal human urine; (b) 2-ME treatment results in the extended, selective induction and stabilization of wild-type p53 protein necessary for apoptosis; (c) the effect of such treatment is restricted specifically to cancer cells and has no effect on normal bronchial epithelial cells, thus increasing the therapeutic index; and (d) 2-ME is effective *in vivo*, even when administered p.o. Interestingly, 2-ME has been reported to induce apoptosis in transformed cells but not in normal cells (21). Thus, it provides a potential means for inducing programmed cell death in aggressive pancreatic cancer cells. This might be of clinical interest because there are few therapeutic options for pancreatic cancer, besides surgery. 2-ME induction of apoptosis in human pancreatic cancer cells, therefore, holds promise as a therapy for pancreatic cancer.

## References

- Gelbke, H. P., and Knuppen, R. The excretion of five different 2-hydroxyoestrogen monomethyl ethers in human pregnancy urine. *J. Steroid Biochem.*, 7: 457–463, 1976.
- Fotsis, T., Zhang, Y., Pepper, M. S., Adlercreutz, H., Montesano, R., Nawroth, P. P., and Schwelgerer, L. The endogenous oestrogen metabolite 2-methoxyestradiol inhibits angiogenesis and suppresses tumour growth. *Nature (Lond.)*, 368: 237–239, 1994.
- Zhu, B. T., and Conney, A. H. Is 2-Methoxyestradiol an endogenous estrogen metabolite that inhibits mammary carcinogenesis? *Cancer Res.*, 58: 2269–2277, 1998.
- Mukhopadhyay, T., and Roth, J. A. Induction of apoptosis in human lung cancer cells after wild-type p53 activation by methoxyestradiol. *Oncogene*, 14: 379–384, 1997.
- Raff, M. C., Barres, B. A., Burne, J. F., Coles, H. S., Ishizaki, Y., and Jacobson, M. D. Programmed cell death and the control of cell survival: lessons from the nervous system. *Science (Washington DC)*, 262: 695–700, 1993.
- Thompson, C. B. Apoptosis in the pathogenesis and treatment of disease. *Science (Washington DC)*, 267: 1456–1462, 1995.
- Vaux, D. L., and Strasser, A. The molecular biology of apoptosis. *Proc. Natl. Acad. Sci. USA*, 93: 2239–2244, 1996.
- Yue, T. L., Wang, X., Loudon, C. S., Gupta, S., Pillarisetti, K., Gu, J. L., Hart, T. K., Lysko, P. G., and Feuerstein, G. Z. 2-Methoxyestradiol, an endogenous estrogen metabolite, induces apoptosis in endothelial cells and inhibits angiogenesis: possible role for stress-activated protein kinase signaling pathway and Fas expression. *Mol. Pharmacol.*, 51: 951–962, 1997.
- Mukhopadhyay, T., and Roth, J. A. Superinduction of wild-type p53 protein after 2-methoxyestradiol treatment of Ad5p53-transduced cells induces tumor cell apoptosis. *Oncogene*, 17: 241–246, 1998.
- Elsasser, H. P., Lehr, U., Agricola, B., and Kern, H. F. Establishment and characterization of two cell lines with different grades of differentiation derived from one primary human pancreatic adenocarcinoma. *Virchows Arch.*, 61: 295–306, 1992.
- Elsasser, H. P., Lehr, U., Agricola, B., and Kern, H. F. Structural analysis of a new highly metastatic cell line PaTu 8902 from a primary human pancreatic adenocarcinoma. *Virchows Arch.*, 64: 201–207, 1993.
- Fujiwara, T., Grimm, E. A., Mukhopadhyay, T., Zhang, W. W., Owen-Schaub, L. B., and Roth, J. A. Induction of chemosensitivity in human lung cancer cells *in vivo* by adenoviral-mediated transfer of the wild-type p53 gene. *Cancer Res.*, 54: 2287–2291, 1994.
- Chen, X., Ko, L. J., Jayaraman, L., and Prives, C. p53 levels, functional domains, and DNA damage determine the extent of the apoptotic response of tumor cells. *Genes Dev.*, 10: 2438–2451, 1996.

14. Ronen, D., Schwartz, D., Teitz, Y., Goldfinger, N., and Rotter, V. Induction of HL-60 cells to undergo apoptosis is determined by high levels of wild-type p53 protein whereas differentiation of the cells is mediated by lower p53 levels. *Cell Growth Differ.*, 7: 21–30, 1996.
15. Merriam, G. R., MacLusky, N. J., Picard, M. K., and Naftolin, F. Comparative properties of the catechol estrogens. I. Methylation by catechol-O-methyltransferase and binding to cytosol estrogen receptors. *Steroids*, 36: 1–11, 1980.
16. Folkman, J., and Shing, Y. Angiogenesis. *J. Biol. Chem.*, 267: 10931–10934, 1992.
17. Fan, T. P., Jaggar, R., and Bicknell, R. Controlling the vasculature: angiogenesis, anti-angiogenesis and vascular targeting of gene therapy. *Trends Pharmacol. Sci.*, 16: 57–66, 1995.
18. Folkman, J. What is the evidence that tumors are angiogenesis dependent? *J. Natl. Cancer Inst.*, 82: 4–6, 1990.
19. Tishler, R. B., Lamppu, D. M., Park, S., and Price, B. D. Microtubule-active drugs taxol, vinblastine, and nocodazole increase the levels of transcriptionally active p53. *Cancer Res.*, 55: 6021–6025, 1995.
20. Attalla, H., Makela, T. P., Adlercreutz, H., and Andersson, L. C. 2-Methoxyestradiol arrests cells in mitosis without depolymerizing tubulin. *Biochem. Biophys. Res. Commun.*, 228: 467–473, 1996.
21. Seegers, J. C., Lottering, M. L., Grobler, C. J. S., Vanpapendorf, D. H., Habbersett, R. C., Shou, Y. L., and Lehnert, B. E. The mammalian metabolite, 2-methoxyestradiol, affects p53 levels and apoptosis induction in transformed cells but not in normal cells. *J. Steroid Biochem. Mol. Biol.*, 62: 253–267, 1997.