Cancer Science
The official journal of the Japanese Cancer Association

Antitumor effects of α-bisabolol against pancreatic cancer

Takashi Seki,[1] Toshio Kokuryo,[1,3] Yukihiro Yokoyama,[1] Hisanori Suzuki,[2] Keita Itatsu,[1] Akifumi Nakagawa,[1] Tetsushi Mizutani,[1] Takashi Miyake,[1] Masanori Uno,[1] Kohei Yamauchi[1] and Masato Nagino[1]

[1]Division of Surgical Oncology, Department of Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan; [2]Department of Sciences of Life and Reproduction, Section of Biochemistry, University of Verona, Verona, Italy

(Received March 21, 2011/Revised June 30, 2011/Accepted August 16, 2011/Accepted manuscript online August 28, 2011/Article first published online September 26, 2011)

In the present study, we investigated whether α-bisabolol, a sesquiterpene alcohol present in essential oils derived from a variety of plants, has antitumor effects against pancreatic cancer. α-Bisabolol induced a decrease in cell proliferation and viability in pancreatic cancer cell lines (KLM1, KP4, Panc1, MIA Paca2), but not in pancreatic epithelial cells (ACBRI515). α-Bisabolol treatment induced apoptosis and suppressed Akt activation in pancreatic cancer cell lines. Furthermore, α-bisabolol treatment induced the overexpression of early growth response-1 (EGR1), whereas EGR1 siRNA decreased the α-bisabolol-induced cell death of KLM1 cells. Tumor growth in both subcutaneous and peritoneal xenograft nude mouse models was significantly inhibited by intragastric administration of 1000 mg/kg of α-bisabolol, once a week for three weeks. The results indicate that α-bisabolol could be a novel therapeutic option for the treatment of pancreatic cancer. (*Cancer Sci* 2011; 102: 2199–2205)

α-Bisabolol [1-methyl-4(1,5-dimethyl-1-hydroxhex-4(5)-enyl)-cyclohexen-1] is a sesquiterpene alcohol found in essential oils derived from a variety of plants, including chamomile (*Chamomilla recutita* L.),[1] salvia (*Salvia runcinata*),[2] *Plinia cerrocampanensis*,[3] among others. α-Bisabolol is non-toxic (LD_{50} 13–14 g/kg of body weight, orally, in adult mice and rats)[4–6] and is known to possess anti-inflammatory,[7,8] antibiotic,[9] gastroprotective,[10] antioxidant,[11] and antimutagenic[12] effects. In addition, recent reports have shown that α-bisabolol inhibits the growth and survival of glioblastoma, pancreatic cancer, prostate cancer, breast cancer, and liver cancer *in vitro*;[5,13,14] in contrast, α-bisabolol has shown no toxicity against normal cells.[5] The molecular mechanisms underlying α-bisabolol-mediated cytotoxicity to malignant tumor cells remain largely unclear and there are few reports demonstrating the effects of α-bisabolol on malignant tumors in animal models.[15]

In the present study we investigated the effects of α-bisabolol *in vitro* and *in vivo* using pancreatic cancer cell lines. We observed that exposure of pancreatic cancer cell lines to α-bisabolol resulted in suppression of growth and cell death *in vitro*. We also found that the tumor suppressive effects of α-bisabolol could be explained, in part, by suppression of Akt phosphorylation and overexpression of early growth response-1 (EGR1). Moreover, intragastric administration of α-bisabolol suppressed tumor growth in tumor-bearing mice.

Materials and Methods

Materials. α-Bisabolol was purchased from Sigma-Aldrich (St Louis, MO, USA), as was anti-β-actin antibody. Anti-poly-(ADP-ribose) polymerase (PARP), anti-Akt, anti-phosphorylated (p-) Akt (Ser^{473}), anti-p-Akt (Thr^{308}), anti-3-phosphoinositide-dependent kinase 1 (PDK1), anti-phosphatidylinositol 3-kinase (PI3K) (p110), anti-PI3K (p85), anti-mammalian target of rapamycin (mTOR), anti-Rictor, and anti-EGR1 antibodies were obtained from Cell Signaling Technology (Danvers, MA, USA).

Cell culture. KLM1 and Panc1 human pancreatic cancer cell lines were kindly provided by the Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer, Tohoku University. The MIA Paca2 and KP4 human pancreatic cancer cell lines were obtained from Riken BioResource Center (Ibaraki, Japan). The KLM1, KP4, and Panc1 cells were maintained in RPMI 1640 (Invitrogen Life Technologies, Carlsbad, CA, USA) supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. The MIA Paca2 cells were maintained in Dulbecco's modified Eagle's medium (Sigma-Aldrich) with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. The ACBRI515 human pancreatic epithelial cells were obtained from Applied Cell Biology Research Institute (Kirkland, WA, USA) and maintained in CSC medium (Cell Systems, Kirkland, WA, USA) with 100 U/mL penicillin and 100 μg/mL streptomycin. All cell lines were incubated at 37°C in a humidified atmosphere containing 5% CO_2.

Real-time RT-PCR. To validate changes in gene expression, quantitative real-time RT-PCR analysis was performed using a Prism 7300 sequence detection system (Applied Biosystems, Foster City, CA, USA). Total RNA was isolated from cell lines using RNeasy Mini kits (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The cDNA was generated from total RNA samples using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems). Each reaction was performed in 20 μL reaction mixture containing TaqMan universal PCR master mix according to the manufacturer's instructions (Applied Biosystems). The TaqMan probe and primer for EGR1 (assay identification no. Hs00152928_m1*) were purchased from Applied Biosystems; 18s rRNA (assay identification no. Hs99999901_s1; Applied Biosystems) was used as an internal control.

Western blot analysis. Whole cell extracts and tumor tissues were prepared by lysing cells in Laemmli sample buffer. An equivalent amount of cell lysate was electrophoresed on SDS–polyacrylamide gels, transferred to PVDF membranes (Immobilon; Millipore, Billerica, MA, USA), and probed with antibodies. Signals were detected using an ECL system (GE Healthcare Life Sciences, Buckinghamshire, UK).

Transfection of siRNA. The EGR1-specific siRNA used in the present study was purchased from Sigma-Aldrich. The siRNA targeting EGR1 sequences were as follows: EGR1 siRNA 2227, 5′-CCAACGACAGCAGUCCCAUTT-3′ (sense) and 5′-AUGGGACUGCUGUCGUUGGTT-3′ (antisense); and EGR1 siRNA 2228, 5′-GACCUGAAGGCCCUCAAUATT-3′ (sense) and 5′-UAUUGAGGGCCUUCAGGUCTT-3′ (antisense). The sequence of the control siRNA (Qiagen) was 5′-UUCUCCGAACGUGUCACGUTT-3′ (sense) and 5′-ACGUGACACGUUCGGAGA-

[3]To whom correspondence should be addressed.
E-mail: kokuryoh@med.nagoya-u.ac.jp

doi: 10.1111/j.1349-7006.2011.02082.x

ATT-3′ (antisense). Cells were transfected with 1 nM siRNA using HiperFect Transfection Reagent (Qiagen) according to the manufacturer's instructions.

Cell proliferation assay. Cell proliferation was determined using the MTT assay. Briefly, KLM1, KP4, Panc1, MIA Paca2, and ACBRI515 cells were seeded at a density of 2000 cells/well in 96-well plates. After incubation overnight at 37°C and 5% CO_2, the medium was removed and replaced with fresh medium containing different concentrations (0–250 μM) of α-bisabolol (diluted from a 5 mg/mL stock solution using ethanol);[5] this time point was set as 0 h. At the indicated time points (0, 24, 48, 72 h), 10 μL MTT solution (Tetra Color One; Seikagaku Biobusiness, Tokyo, Japan) was added to each well and cells were incubated for 1.5 h, after which the absorbance of each well was measured at 450 and 630 nm using a microplate reader.

Cell viability assay. Cell death was determined by the Trypan blue dye exclusion test. Live cells that have intact membranes exclude the dye, whereas dead cells do not and are thus stained. Briefly, KLM1, KP4, Panc1, MIA Paca2, and ACBRI515 cells were seeded at a density of 2×10^5 cells/well in six-well plates. After incubation overnight at 37°C and 5% CO_2, the medium was removed and replaced with fresh medium containing different concentrations (0–250 μM) of α-bisabolol (diluted from a 5 mg/mL stock solution using ethanol);[5] this time point was set as 0 h. At the indicated time points (24, 48, 72 h), floating and attached cells were collected and stained with 1% Trypan blue. Counts were performed using a Countess Automated Cell Counter (Invitrogen) in duplicate wells.

Colony formation assay. Anchorage-independent cell growth was determined using a CytoSelect 96-well cell transformation assay (soft agar colony formation; Cell Biolabs, San Diego, CA, USA) according to the manufacturer's instructions.

TUNEL assay. Cells were treated with 250 μM of α-bisabolol for 6 h before being fixed to glass slides with a Cyto-Tek cytocentrifuge (300*g*, 4°C and 5 min; Sakura Finetek, Torrance, CA, USA). TUNEL assays were performed using the DeadEnd Colorimetric TUNEL system (Promega, Madison, WI, USA) according to the manufacturer's instructions.

Histological evaluation. Tissues from nude mice were examined histologically. Tumor samples were fixed immediately in neutral buffered formalin and embedded in paraffin. Immunohistochemistry was used to detect the expression of Akt, p-Akt, and EGR1 in tissue sections. Slides were prepared using a Discovery XT automated slide preparation system (Ventana Medical Systems, Tucson, AZ, USA). Before staining, paraffin-embedded sections were blocked with 5% non-fat milk. The staining procedure was performed according to the manufacturer's instructions (Ventana Medical Systems). Anti-Akt, anti-p-Akt (Ser^{473}), anti-p-Akt (Thr^{308}; Abcam, Cambridge, UK), and EGR1 antibodies were diluted in Discovery Ab diluent (Ventana Medical Systems).

Antitumor efficacy in animal models. Male BALB/c nude mice (7 weeks old and weighing 20–25 g) were purchased from SLC Japan (Nagoya, Japan). Mice were kept in a temperature- and humidity-controlled environment under a 12-h light–dark cycle and had free access to water and food at all times. Animal experiments were performed in compliance with the guidelines of the Institute for Laboratory Animal Research, Nagoya University Graduate School of Medicine.

Subcutaneous xenograft model. Mice were inoculated with KLM1 and KP4 cells (1×10^7), injected subcutaneously into the femoral area.[16] After 1 week, α-bisabolol (1000 mg/kg), diluted in olive oil (total volume 200 μL), was administered intragastrically using an orogastric tube once a week for 3 weeks. Intragastric administration of an equal volume of olive oil alone was used as a control. The antitumor efficacy was assessed by estimating tumor volume (in mm^3), calculated as $(L \times W^2)/2$, where L is tumor length (in mm) and W is tumor width (in mm).[17,18]

Peritoneal xenograft model. KLM1 cells (1×10^7) were injected into the intraperitoneal cavity of mice. After 1 week, mice were treated with α-bisabolol or olive oil alone as described for the subcutaneous xenograft model. Antitumor efficacy was assessed on the basis of the total weight of the intraperitoneal tumor.

Statistical analysis. Data are presented as the mean ± SEM. The significance of differences between experimental values was assessed using Student's *t*-test and $P < 0.05$ was considered significant.

Results

Effects of α-bisabolol on the viability of pancreatic cancer cells. We first examined the antiproliferative effects of α-bisabolol on pancreatic cancer cell lines (KLM1, KP4, Panc1, MIA Paca2) and pancreatic epithelial cells (ACBRI515) using the MTT assay (Fig. 1a). Cells were treated with 0–250 μM α-bisabolol for 24, 48, and 72 h. Because α-bisabolol is a highly lipophilic agent and because only 2.5% of it is dissolved in medium even if it is diluted with ethanol,[5] the estimated concentrations following administration of 0–250 μM α-bisabolol treatment were 0–6.25 μM, respectively. All concentrations of α-bisabolol referred to hereafter are the estimated concentration (0–6.25 μM). α-Bisabolol markedly suppressed the proliferation of KLM1 and KP4 cells at a concentration of 5 μM, and that of Panc1 and MIA Paca2 cells at a concentration of 6.25 μM. Interestingly, α-bisabolol had no significant effect on the proliferation of ACBRI515 cells.

In the present study, the Trypan blue dye exclusion test was used to confirm the effects of α-bisabolol on the viability of pancreatic cancer cell lines and pancreatic epithelial cells (Fig. 1b). After 48 h, 5 μM α-bisabolol induced >50% cell death in all four pancreatic cancer cell lines. In contrast, the same concentration induced only 18% cell death in ACBRI515 cells.

We also performed colony formation assays to confirm the effects of α-bisabolol on the anchorage-independent cell growth of pancreatic cancer cell lines and pancreatic epithelial cells (Fig. 1c,d). α-Bisabolol treatment suppressed the growth of KLM1 and KP4 cells, but not the growth of ACBRI515 cells. These results indicate that α-bisabolol has selective efficacy towards pancreatic cancer cells.

α-Bisabolol induced apoptosis of pancreatic cancer cells. α-Bisabolol has been reported to induce apoptosis in malignant tumor cells.[5,6,13,14,19] Therefore, in the present study, we examined the induction of apoptosis in pancreatic cancer cells after α-bisabolol treatment using the TUNEL assay (Fig. 2a). More TUNEL-positive cells were seen in pancreatic cancer cell lines treated with α-bisabolol compared with control cells. Furthermore, the expression of cleaved PARP, one of the key mediators of apoptosis,[20,21] was markedly increased in pancreatic cancer cell lines after α-bisabolol treatment (Fig. 2b). In contrast, α-bisabolol did not induce apoptosis of pancreatic epithelial cells.

Suppression of Akt phosphorylation by α-bisabolol *in vitro*. The link between Akt inactivation and apoptosis has been reported in human cancers.[22–25] Therefore, we next examined Akt activation using western blot analysis (Fig. 3a). α-Bisabolol reduced phosphorylation of Thr^{308} and Ser^{473} of Akt in all four pancreatic cancer cell lines. Akt phosphorylation at Thr^{308} is regulated by PDK1,[26] whereas phosphorylation at Ser^{473} is regulated by mTOR complex 2 (mTORC2), which is a complex of mTOR and Rictor.[26,27] Phosphorylation of both Thr^{308} and Ser^{473} is required for full activation of Akt.[26] Furthermore, Akt and PDK1 are regulated by PI3K.[26,28–30] Following α-bisabolol treatment, PI3K (either p110 or p85 subtype) and PDK1 were suppressed in all four cell lines. Although mTOR levels did not

doi: 10.1111/j.1349-7006.2011.02082.x

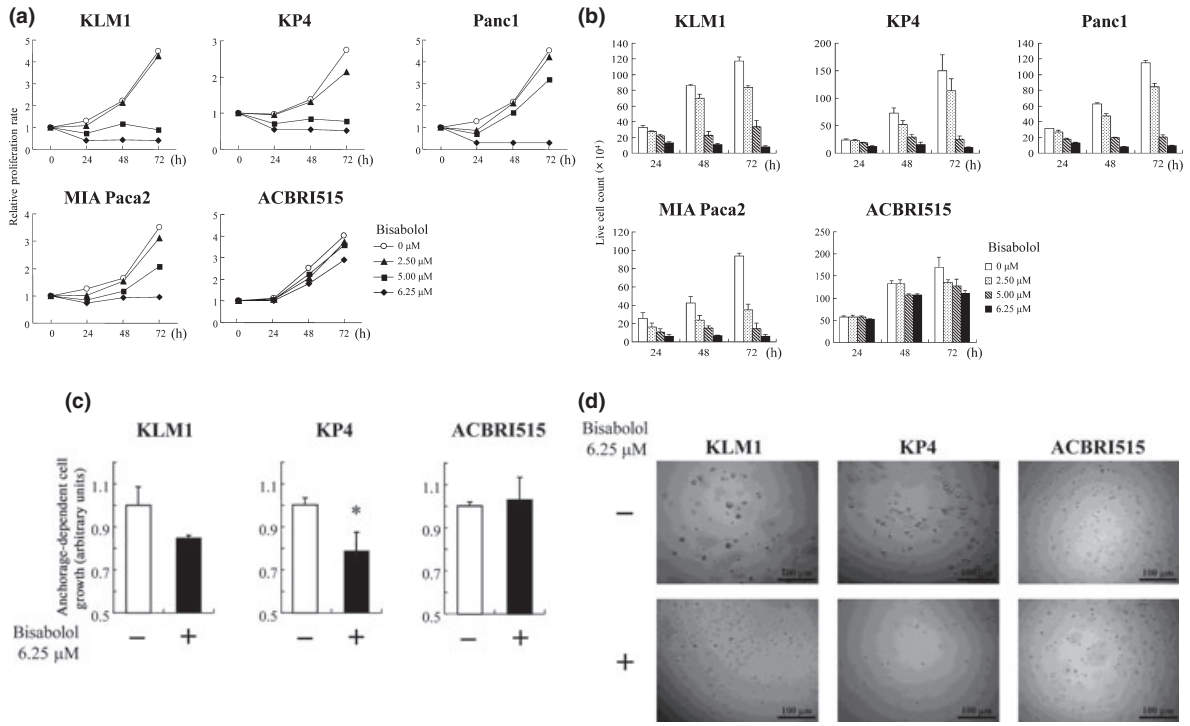

Fig. 1. Human pancreatic cancer cell lines (KLM1, KP4, Panc1, MIA Paca2) and human pancreatic epithelial cells (ACBRI515) were treated with different concentrations of α-bisabolol for 24, 48, and 72 h. (a) Cell proliferation was determined using the MTT assay. Data are relative values to the zero time point. Each point is the mean of six replicate wells. (b) Cell viability determined by the Trypan blue dye exclusion, showing the live cell count. Results are the mean ± SEM of four independent experiments. (c,d) Anchorage-independent cell growth of three cell lines (KLM1, KP4, ACBRI515) determined using the colony formation assay. Cells were treated with 6.25 μM α-bisabolol for 10 days. (c) Results are shown as the mean ± SEM of four independent experiments. *$P < 0.05$. (d) Representative photographs.

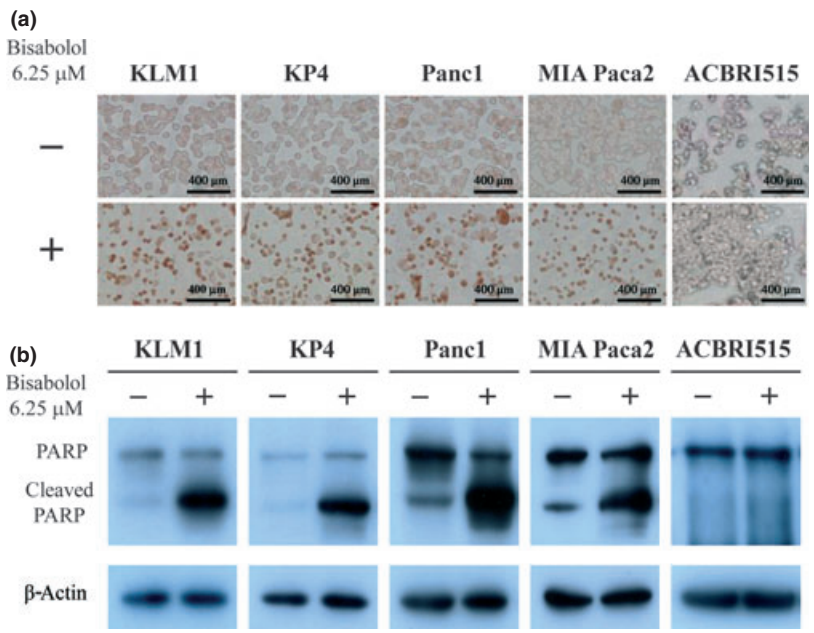

Fig. 2. (a) Human pancreatic cancer cell lines (KLM1, KP4, Panc1, MIA Paca2) and human pancreatic epithelial cells (ACBRI515) were treated with (+) or without (–) 6.25 μM α-bisabolol for 6 h and then subjected to a TUNEL assay. (b) Cleavage of poly(ADP-ribose) polymerase (PARP), as assessed by Western blot analysis with an anti-PARP antibody. Five cell lines were treated with (+) or without (–) 6.25 μM α-bisabolol for 6 h. β-Actin was used as an internal control. Representative results are shown.

differ following α-bisabolol treatment, Rictor was suppressed in all four cell lines (Fig. 3b). In contrast, α-bisabolol treatment did not induce any changes in the levels of these protein in pancreatic epithelial cells. These results indicate that Akt activation is one of the targets of α-bisabolol in its activity against pancreatic cancer.

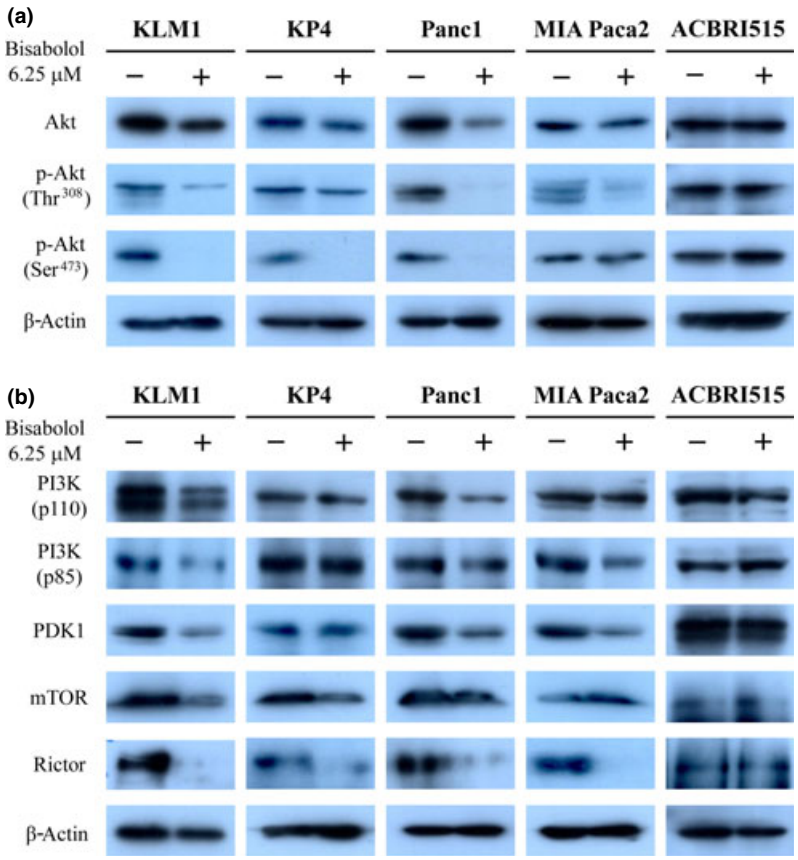

Fig. 3. Human pancreatic cancer cell lines (KLM1, KP4, Panc1, MIA Paca2) and human pancreatic epithelial cells (ACBRI515) were treated with (+) or without (–) 6.25 μM α-bisabolol for 6 h. Western blot analysis was used to determine levels of (a) Akt and phosphorylated (p-) Akt, as well as (b) phosphatidylinositol 3-kinase (PI3K), 3-phosphoinositide-dependent kinase 1 (PDK1), mammalian target of rapamycin (mTOR), and mTOR complex 2 (mTORC2), a complex of mTOR and Rictor. β-Actin was used as an internal control.

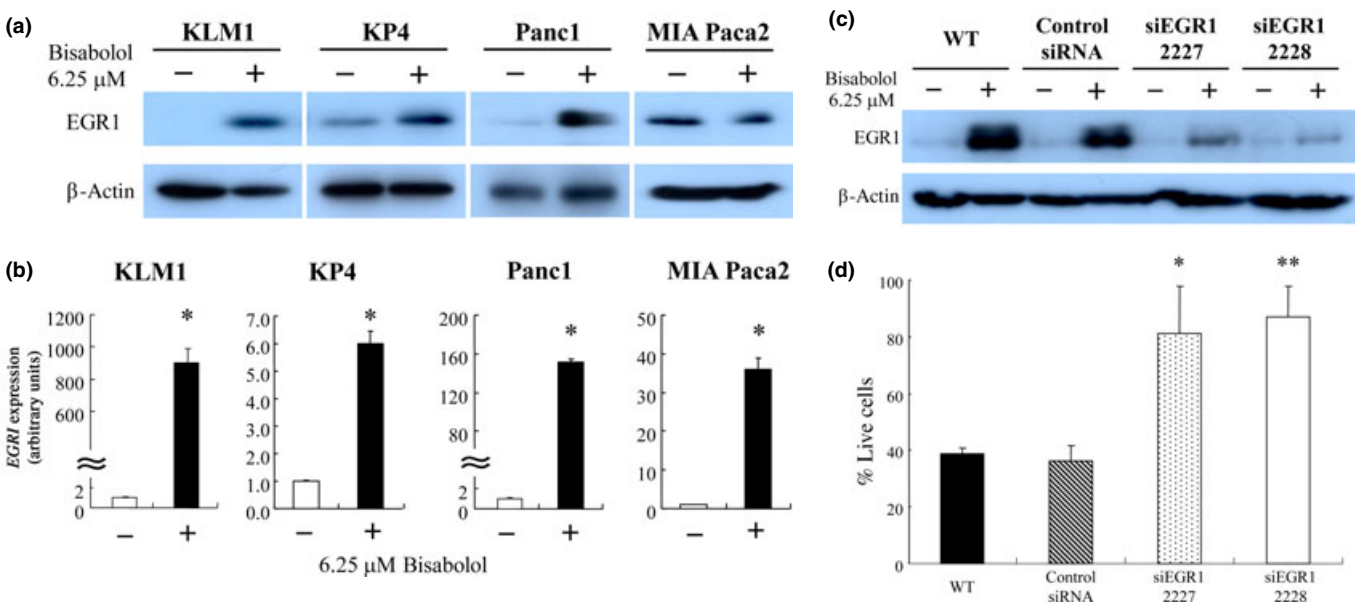

Fig. 4. (a) Human pancreatic cancer cell lines (KLM1, KP4, Panc1, MIA Paca2) were treated with (+) or without (–) 6.25 μM α-bisabolol for 6 h, and early growth response-1 (EGR1) expression was assessed by western blot analysis. β-Actin was used as an internal control. (b) Expression of the *EGR1* gene in pancreatic cancer cell lines with (+) or without (–) 6.25 μM α-bisabolol treatment for 3 h, determined quantitative real-time RT-PCR. Data are the mean ± SEM. $^{*}P < 0.01$ compared with values obtained in the absence of bisabolol. (c) KLM1 cells were transfected with EGR1 or control siRNA and were treated with (+) or without (–) α-bisabolol for 6 h, after which EGR1 expression was determined by western blot analysis. β-Actin was used as an internal control. WT, wild type. (d) Viability of KLM1 cells were transfected with EGR1 siRNA determined by the Trypan blue dye exclusion test. Data are the mean ± SEM and are presented as the percentage of live cells, (no. live cells + bisabolol)/(no. live cells – bisabolol) × 100, with or without α-bisabolol (6.25 μM). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with WT.

doi: 10.1111/j.1349-7006.2011.02082.x

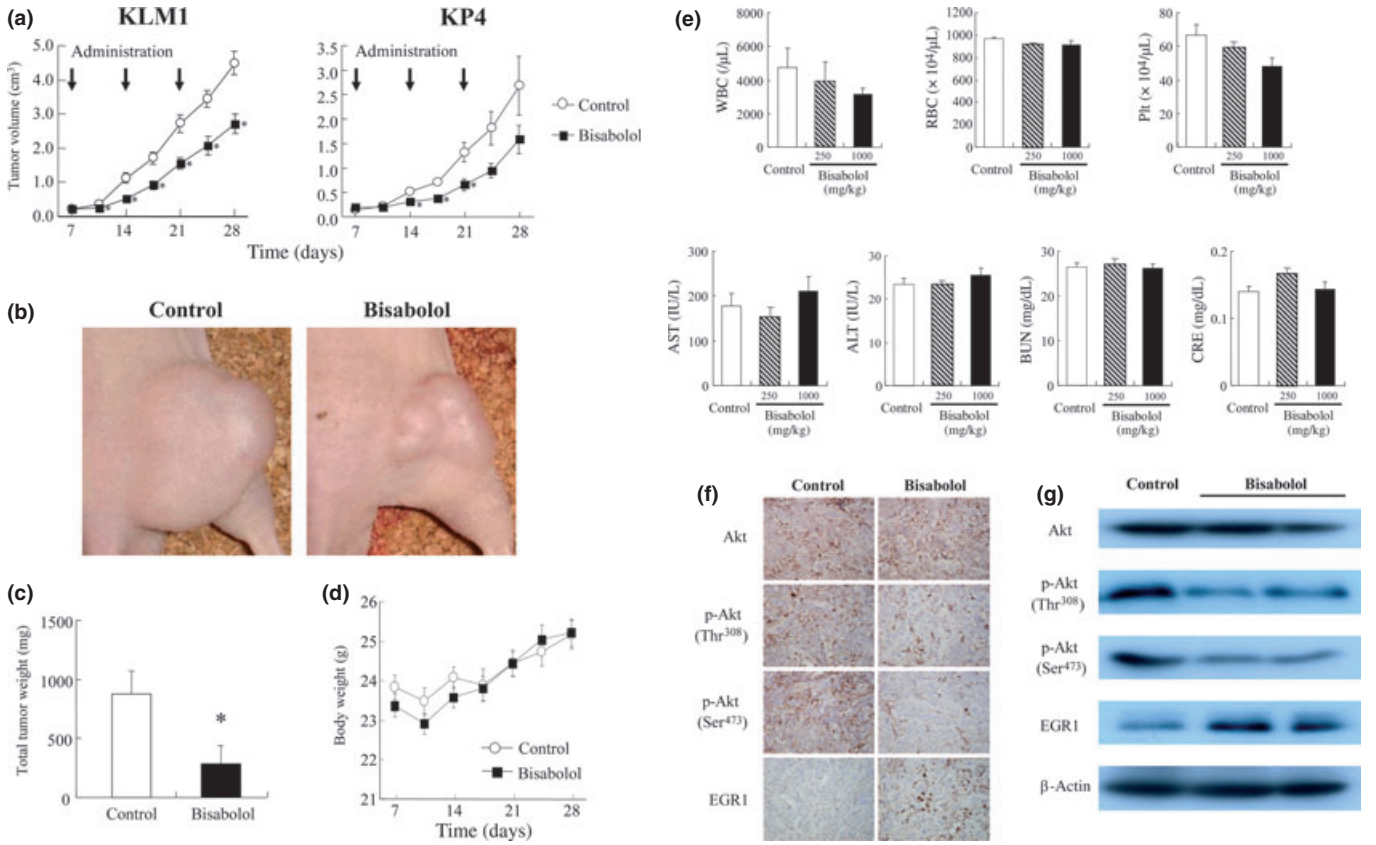

Fig. 5. Effects of intragastric administration of α-bisabolol (21–27 mg/mouse) on the growth of pancreatic cancer in nude mouse models. Olive oil was used as a control. (a) Tumor volume of KLM1 and KP4 subcutaneous xenograft (n = 12 and 5, respectively). One week after tumor inoculation, intragastric administration of α-bisabolol was initiated. Nude mice bearing KLM1 tumors were treated with α-bisabolol once a week for 3 weeks, as indicated by the arrows. Tumor volumes were measured twice a week for 28 days. Data are the mean ± SEM. *$P < 0.05$ compared with control. (b) Representative photographs of the KP4 xenograft 1 week after the final administration of olive oil (control) or α-bisabolol. (c) Total weight of intraperitoneal KLM1 tumors 1 week after the final administration of α-bisabolol (n = 6). One week after intraperitoneal injection of KLM1 cells, mice were treated as in the subcutaneous xenograft model. Data are the mean ± SEM. *$P < 0.05$ compared with control. (d) Changes in body weight in KLM1- or KP4-inoculated mice treated with α-bisabolol or olive oil as a control. (e) White blood cell (WBC) counts, red blood cell (RBC) counts, thrombocytes (Plt), liver function (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]), and renal function (blood urea nitrogen [BUN] and creatinine [CRE]) in mice treated with α-bisabolol (250 or 1000 mg/kg) or olive oil as a control. Data are the mean ± SEM. (f,g) Expression of Akt, phosphorylated (p-) Akt, and early growth response-1 (EGR1) in subcutaneously implanted KLM1 cells after daily intragastric administration of α-bisabolol for 3 days, as assessed by western blot analysis (f) and immunohistochemistry (g). β-Actin was used as an internal control. (Original magnification ×200.)

Induction of EGR1 overexpression by α-bisabolol treatment. It has been reported that EGR1 is a tumor suppressor, because its overexpression markedly inhibits tumor cell growth[31,32] in an Akt-dependent manner.[33] Therefore, we hypothesized that the antitumor activity of α-bisabolol was related to EGR1 activation. Although only a small amount of EGR1 was expressed in KLM1, KP4, and Panc1 cells without α-bisabolol treatment, high levels of EGR1 expression were detected after 6 h treatment with 6.25 μM α-bisabolol (Fig. 4a). Furthermore, the gene expression of EGR1 was significantly higher in all four cell lines after 3 h treatment with 6.25 μM α-bisabolol compared with control (Fig. 4b). To further elucidate the functional mechanism underlying α-bisabolol-induced EGR1 overexpression, we performed *in vitro* Trypan blue dye exclusion tests using EGR1 siRNA. Silencing of EGR1 (Fig. 4c) partially abolished cell death induced by α-bisabolol (Fig. 4d). These results indicate that EGR1 activation is one of the mechanisms underlying the antitumor effects of α-bisabolol against pancreatic cancer.

Antitumor effects of intragastric α-bisabolol in nude mouse models. Because α-bisabolol is a non-toxic natural product, it may be useful as an oral anticancer drug. Therefore, we examined the effects of intragastric administration of α-bisabolol on the growth of pancreatic cancer cells using nude mouse xenograft models. Mice were inoculated subcutaneously with either KLM1 or KP4. One week after tumor inoculation, α-bisabolol was administered (21–27 mg/mouse) diluted in olive oil once a week for a total of 3 weeks. Tumor volume was measured twice a week for 28 days. As shown in Figure 5(a,b), mean tumor volumes of α-bisabolol-treated mice were significantly lower than those in control mice. We further examined the effects of α-bisabolol on the growth of intraperitoneally implanted pancreatic cancer cells (Fig. 5c). One week after mice had been injected with KLM1 cells, intragastric administration of α-bisabolol was started, as for the subcutaneous xenograft model. Four weeks later, the total weight of the intraperitoneal tumors was measured. The mean tumor weight of the mice treated with α-bisabolol was significantly lower than that of control mice. Surprisingly, two mice treated with α-bisabolol had no intraperitoneal tumors. We observed no toxicity, such as reductions in bodyweight (Fig. 5d) or red blood cell counts and liver and/or renal dysfunction, following α-bisabolol administration to mice

(Fig. 5e). Although white blood cell and thrombocyte counts decreased in dose-dependent manner following α-bisabolol administration (Fig. 5e), the differences did not reach statistical significance compared with the control group. Therefore, we considered that the intragastric administration of 1000 mg/kg α-bisabolol was tolerable. There were no significant differences in the expression of p-Akt and EGR1 in KLM1 tumors between the control and α-bisabolol-treated mice after 3 weeks treatment (data not shown). However, there was a marked difference in the expression of p-Akt (suppression) and EGR1 (overexpression) in KLM1 tumors after daily administration of α-bisabolol for 3 days (Fig. 5f). These changes were similar to those observed *in vitro*. We also confirmed the suppression of Akt phosphorylation and the overexpression of EGR1 in the implanted tumors using western blot analysis (Fig. 5g).

Discussion

Pancreatic cancer remains one of the most devastating malignant cancers. Approximately one-third of patients are diagnosed as having locally advanced disease, and half of all patients already have metastases at the time of diagnosis. Even in patients with resectable disease, the long-term outcome remains unsatisfactory because of early recurrence after resection.[34] Therefore, alternative treatments are required to improve the prognosis of patients with pancreatic cancer.

In the present study, we demonstrated that α-bisabolol inhibited the proliferation of pancreatic cancer cells and induced their apoptosis *in vitro*. α-Bisabolol administration was effective against pancreatic cancer without causing any major side effects. These observations highlight a possible role for α-bisabolol as a novel antitumor drug for the treatment of pancreatic cancer. α-Bisabolol induces apoptosis by way of the intrinsic pathway,[5] involving lipid rafts, Bid, and mitochondrial pore permeability.[6,19] Another report has shown that α-bisabolol induces apoptosis by the Fas- and mitochondrial-related pathways, involving p53 and nuclear factor-κB.[14] However, the molecular mechanism underlying α-bisabolol cytotoxicity in malignant tumor cells remains largely unclear. In the present study, we found that α-bisabolol inhibited Akt activation, as well as the expression of its upstream signals (PI3K, PDK1, and mTORC2). Akt is one of the most frequently activated serine/threonine kinases in pancreatic cancer[24,25,35,36] and Akt activation has been reported to be associated with a poorer prognosis in pancreatic cancer patients.[37,38] The activation of Akt causes resistance to apoptosis, increased cell growth, and increased cellular metabolism.[22,23,26,39] These effects may render the tumor cells resistant to therapy. Therefore, Akt-targeted molecular therapy has become an area of intense research in an effort to establish more effective therapies against pancreatic cancer. In this regard, our findings suggest that α-bisabolol could be a promising Akt inhibitor in pancreatic cancer.

We also found that α-bisabolol induced the expression of EGR1 in pancreatic cancer. It is known that EGR1 is a tumor suppressor in the zinc finger transcription factor family and expression of EGR1 is suppressed in many human cancers.[40] Furthermore, it has been reported that Akt activation is associated with the suppression of EGR1 expression.[33,41] Although the precise mechanism involved in the regulation of EGR1 remains unclear, our results indicate that the induction of EGR1 protein by α-bisabolol is associated, at least in part, with Akt inhibition in pancreatic cancer. The suppression of EGR1 enhanced cell survival, consistent with previously published reports.[31,32] Nevertheless, silencing of EGR1 by siRNA did not completely inhibit α-bisabolol-induced apoptosis. Interestingly, α-bisabolol inhibited the growth of MIA Paca2 cells, despite not having any significant effect on EGR1 protein levels in this cell line. As shown in Figure 4, the *EGR1* gene was overexpressed within 3 h of α-bisabolol treatment; however, EGR1 protein was not upregulated even at 6 h after treatment in MIA Paca2 cells. These results indicate that there is a regulatory mechanism involved in the effects of α-bisabolol in MIA Paca2 cells, such as the degradation of overexpressed EGR1. There may be other complicated mechanisms involved in the antitumor effects of α-bisabolol in pancreatic cancer. Further experiments are required to clarify these mechanisms.

In summary, our study has shown for the first time that α-bisabolol can suppress the proliferation and survival of pancreatic cancer cells by inhibiting Akt activity and upregulating EGR1 in pancreatic cancer. These data provide a novel explanation for the anticancer properties of α-bisabolol in pancreatic cancer.

Disclosure Statement

The authors have no conflict of interest.

References

1 Orav A, Raal A, Arak E. Content and composition of the essential oil of *Chamomilla recutita* (L.) Rauschert from some European countries. *Nat Prod Res* 2010; **24**: 48–55.
2 Kamatou GP, Makunga NP, Ramogola WP, Viljoen AM. South African *Salvia* species: a review of biological activities and phytochemistry. *J Ethnopharmacol* 2008; **119**: 664–72.
3 Vila R, Santana AI, Perez-Roses R *et al.* Composition and biological activity of the essential oil from leaves of *Plinia cerrocampanensis*, a new source of alpha-bisabolol. *Bioresour Technol* 2010; **101**: 2510–4.
4 Bhatia SP, McGinty D, Letizia CS, Api AM. Fragrance material review on alpha-bisabolol. *Food Chem Toxicol* 2008; **46** (Suppl 11): S72–6.
5 Cavalieri E, Mariotto S, Fabrizi C *et al.* alpha-Bisabolol, a nontoxic natural compound, strongly induces apoptosis in glioma cells. *Biochem Biophys Res Commun* 2004; **315**: 589–94.
6 Darra E, Abdel-Azeim S, Manara A *et al.* Insight into the apoptosis-inducing action of alpha-bisabolol towards malignant tumor cells: involvement of lipid rafts and Bid. *Arch Biochem Biophys* 2008; **476**: 113–23.
7 Jakovlev V, Isaac O, Thiemer K, Kunde R. [Pharmacological investigations with compounds of chamomile: II. New investigations on the antiphlogistic effects of (−)-alpha-bisabolol and bisabolol oxides.] *Planta Med* 1979; **35**: 125–40. (In German.)
8 Leite GD, Leite LH, Sampaio RD *et al.* (−)-alpha-Bisabolol attenuates visceral nociception and inflammation in mice. *Fitoterapia* 2010; **82**: 208–11.
9 Brehm-Stecher BF, Johnson EA. Sensitization of *Staphylococcus aureus* and *Escherichia coli* to antibiotics by the sesquiterpenoids nerolidol, farnesol, bisabolol, and apritone. *Antimicrob Agents Chemother* 2003; **47**: 3357–60.
10 Moura Rocha NF, Venancio ET, Moura BA *et al.* Gastroprotection of (−)-alpha-bisabolol on acute gastric mucosal lesions in mice: the possible involved pharmacological mechanisms. *Fundam Clin Pharmacol* 2010; **24**: 63–71.
11 Braga PC, Dal Sasso M, Fonti E, Culici M. Antioxidant activity of bisabolol: inhibitory effects on chemiluminescence of human neutrophil bursts and cell-free systems. *Pharmacology* 2009; **83**: 110–5.
12 Gomes-Carneiro MR, Dias DM, De-Oliveira AC, Paumgartten FJ. Evaluation of mutagenic and antimutagenic activities of alpha-bisabolol in the Salmonella/microsome assay. *Mutat Res* 2005; **585**: 105–12.
13 Darra E, Lenaz G, Cavalieri E *et al.* Alpha-bisabolol: unexpected plant-derived weapon in the struggle against tumour survival? *Ital J Biochem* 2007; **56**: 323–8.
14 Chen W, Hou J, Yin Y *et al.* alpha-Bisabolol induces dose- and time-dependent apoptosis in HepG2 cells via a Fas- and mitochondrial-related pathway, involves p53 and NFkappaB. *Biochem Pharmacol* 2010; **80**: 247–54.
15 Costarelli L, Malavolta M, Giacconi R *et al. In vivo* effect of alpha-bisabolol, a nontoxic sesquiterpene alcohol, on the induction of spontaneous mammary tumors in HER-2/neu transgenic mice. *Oncol Res* 2010; **18**: 409–18.
16 Kokuryo T, Senga T, Yokoyama Y, Nagino M, Nimura Y, Hamaguchi M. Nek2 as an effective target for inhibition of tumorigenic growth and peritoneal dissemination of cholangiocarcinoma. *Cancer Res* 2007; **67**: 9637–42.

doi: 10.1111/j.1349-7006.2011.02082.x

17 Yamori T, Sato S, Chikazawa H, Kadota T. Anti-tumor efficacy of paclitaxel against human lung cancer xenografts. *Jpn J Cancer Res* 1997; **88**: 1205–10.
18 Littlejohn JE, Cao X, Miller SD *et al.* Bcl-xL antisense oligonucleotide and cisplatin combination therapy extends survival in SCID mice with established mesothelioma xenografts. *Int J Cancer* 2008; **123**: 202–8.
19 Cavalieri E, Bergamini C, Mariotto S *et al.* Involvement of mitochondrial permeability transition pore opening in alpha-bisabolol induced apoptosis. *FEBS J* 2009; **276**: 3990–4000.
20 Kaufmann SH, Desnoyers S, Ottaviano Y, Davidson NE, Poirier GG. Specific proteolytic cleavage of poly(ADP-ribose) polymerase: an early marker of chemotherapy-induced apoptosis. *Cancer Res* 1993; **53**: 3976–85.
21 Soldani C, Scovassi AI. Poly(ADP-ribose) polymerase-1 cleavage during apoptosis: an update. *Apoptosis* 2002; **7**: 321–8.
22 Luo J, Manning BD, Cantley LC. Targeting the PI3K–Akt pathway in human cancer: rationale and promise. *Cancer Cell* 2003; **4**: 257–62.
23 Hay N. The Akt–mTOR tango and its relevance to cancer. *Cancer Cell* 2005; **8**: 179–83.
24 Hamacher R, Schmid RM, Saur D, Schneider G. Apoptotic pathways in pancreatic ductal adenocarcinoma. *Mol Cancer* 2008; **7**: 64.
25 Arlt A, Müerköster SS, Schäfer H. Targeting apoptosis pathways in pancreatic cancer. *Cancer Lett* 2010; doi:10.1016/j.canlet.2010.10.015 [Epub ahead of print].
26 Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nat Rev Cancer* 2009; **9**: 550–62.
27 Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor–mTOR complex. *Science* 2005; **307**: 1098–101.
28 Cantrell DA. Phosphoinositide 3-kinase signalling pathways. *J Cell Sci* 2001; **114**: 1439–45.
29 Yuan TL, Cantley LC. PI3K pathway alterations in cancer: variations on a theme. *Oncogene* 2008; **27**: 5497–510.
30 Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nat Rev Cancer* 2002; **2**: 489–501.
31 Baron V, Adamson ED, Calogero A, Ragona G, Mercola D. The transcription factor Egr1 is a direct regulator of multiple tumor suppressors including TGFbeta1, PTEN, p53, and fibronectin. *Cancer Gene Ther* 2006; **13**: 115–24.
32 Su L, Cheng H, Sampaio AV, Nielsen TO, Underhill TM. EGR1 reactivation by histone deacetylase inhibitors promotes synovial sarcoma cell death through the PTEN tumor suppressor. *Oncogene* 2010; **29**: 4352–61.
33 Yu J, Zhang SS, Saito K *et al.* PTEN regulation by Akt–EGR1–ARF–PTEN axis. *EMBO J* 2009; **28**: 21–33.
34 Okusaka T, Ito Y, Furuse J *et al.* Current status of chemoradiotherapy for locally advanced pancreatic cancer in Japan. *Int J Clin Oncol* 2008; **13**: 127–31.
35 Schlieman MG, Fahy BN, Ramsamooj R, Beckett L, Bold RJ. Incidence, mechanism and prognostic value of activated AKT in pancreas cancer. *Br J Cancer* 2003; **89**: 2110–5.
36 Bondar VM, Sweeney-Gotsch B, Andreeff M, Mills GB, McConkey DJ. Inhibition of the phosphatidylinositol 3′-kinase–AKT pathway induces apoptosis in pancreatic carcinoma cells *in vitro* and *in vivo*. *Mol Cancer Ther* 2002; **1**: 989–97.
37 Semba S, Moriya T, Kimura W, Yamakawa M. Phosphorylated Akt/PKB controls cell growth and apoptosis in intraductal papillary-mucinous tumor and invasive ductal adenocarcinoma of the pancreas. *Pancreas* 2003; **26**: 250–7.
38 Yamamoto S, Tomita Y, Hoshida Y *et al.* Prognostic significance of activated Akt expression in pancreatic ductal adenocarcinoma. *Clin Cancer Res* 2004; **10**: 2846–50.
39 Brazil DP, Yang ZZ, Hemmings BA. Advances in protein kinase B signalling: AKTion on multiple fronts. *Trends Biochem Sci* 2004; **29**: 233–42.
40 Thiel G, Cibelli G. Regulation of life and death by the zinc finger transcription factor Egr-1. *J Cell Physiol* 2002; **193**: 287–92.
41 Ahn BH, Park MH, Lee YH, Min do S. Phorbol myristate acetate-induced Egr-1 expression is suppressed by phospholipase D isozymes in human glioma cells. *FEBS Lett* 2007; **581**: 5940–4.