Abstract. α-Bisabolol is a plant-derived, oily sesquiterpene alcohol that induces apoptosis of various cancer cells. We previously reported the antiproliferative effects of α-bisabolol on pancreatic cancer cell lines using in vitro and in vivo experiments. However, the effects of α-bisabolol on tumor invasiveness and motility are still unknown. In this study, we demonstrated that α-bisabolol suppressed the invasiveness and motility of a pancreatic cancer cell line. Although Early growth response 1 (EGR1) was involved in antiproliferative effects of α-bisabolol, it had no relationship with the inhibitory effect of α-bisabolol on cellular invasiveness and motility. Polymerase chain reaction analysis revealed that α-bisabolol induced Kisspeptin 1 receptor (KISS1R) in pancreatic cancer cell lines. The inhibition of KISS1R weakened the inhibitory effect of α-bisabolol on invasiveness of pancreatic cancer cells. The results also implied that the inhibitory effects of α-bisabolol on tumor invasiveness and motility are at least partly associated with the activation of KISS1R. However, there is a possibility that other molecular mechanisms of α-bisabolol regulate invasiveness and motility in pancreatic cancer cells. Further investigations are necessary to clarify the precise mechanisms of α-bisabolol activity for clinical application as a novel treatment for pancreatic cancer.

Pancreatic cancer is the fourth-leading cause of cancer-related death in developed countries (1). Even when pancreatic cancer is completely removed by surgical operation, the prognosis is still poor because of metastatic or local recurrence (2). The 5-year survival rate is less than 20% (3, 4). Although chemotherapy and radiotherapy have shown some benefit for the prognosis of patients with pancreatic cancer, the impact of these therapies remains unsatisfactory (5). Therefore, novel treatments are urgently needed to improve the prognosis of the metastasis of pancreatic cancer.

α-Bisabolol is a plant-derived, oily sesquiterpene alcohol. It has several functions, such as being gastric-protective, antimicrobial, and anti-inflammatory. α-Bisabolol also induces apoptosis of glioma cells, acute leukemia cells, and liver carcinoma cells (6-8). We previously reported an antiproliferative effect of α-bisabolol against pancreatic cancer (9). However, the precise role of α-bisabolol concerning the invasiveness and motility of pancreatic cancer is still not fully understood. Furthermore, the key regulatory mechanism of α-bisabolol for invasiveness and motility of cancer cells has never been investigated.

In this study, we investigated the effect of α-bisabolol on invasiveness and motility of pancreatic cancer cells and the mechanism involved.

Materials and Methods

Materials. α-Bisabolol and antibody to β-actin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibodies to early growth response 1 (EGR1) and anti-Kisspeptin 1 receptor (KISS1R) were obtained from Cell Signaling Technology (Danvers, MA, USA).

Cell culture. The human pancreatic cancer cell lines (KL1, KP4 and Panc1) were kindly provided by the Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer, Tohoku University, Japan. The cell lines were cultured in RPMI-1640 medium (Invitrogen Life Technologies, Carlsbad, CA, USA) with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin. All cell lines were incubated at 37°C in a humidified atmosphere containing 5% CO2.

Establishment of shRNA-transfected cells. EGR1 shRNA-transfected KLM1 cells in which EGR1 is constitutively suppressed were established by retrovirus infection. To produce the EGR1 shRNA-transfected KLM1 cell line, oligonucleotides encoding an shRNA specific for human EGR1: 5'-CCAAACGACAGCAGUCCAT-3' (sense) and 5'-AUUGGGACUGCUGUCGUUGT-3' (antisense) were cloned into the pSIREN-RetroQ retroviral vector (Clontech,
Results

α-Bisabolol suppressed the invasiveness and motility of pancreatic cancer cell lines. The effects of α-bisabolol were evaluated using invasion and motility assays in KLM1, KP4, and Panc1 cells. Invasiveness of pancreatic cancer cell lines KLM1, KP4 and Panc1 was significantly suppressed when cells were treated with 1.56 μM of α-bisabolol for 24 h (Figure 1a). The cell-migration assays also showed that α-bisabolol significantly inhibited motility of pancreatic cancer cells (Figure 1b).

EGR1 has no influences in the inhibitory effect of α-bisabolol for invasiveness and motility. EGR1 is a known tumor-suppressor gene of the zinc finger transcription factor family. We previously reported that EGR1 activation was one of the antitumor mechanisms of α-bisabolol against pancreatic cancer (9). To examine the effects of EGR1 on invasiveness and motility in α-bisabolol-treated pancreatic cancer cells, an EGR1 shRNA-transfected KLM1 cell line was established. Western blot analysis demonstrated that EGR1 expression was strongly attenuated in EGR1 shRNA-transfected KLM1 cells after exposure to α-bisabolol 6.25 μM (Figure 2a).

To investigate the effects of α-bisabolol on invasiveness, control shRNA-transfected, and Egr1 shRNA-transfected KLM1 cell lines were treated with 1.56 μM α-bisabolol for 24 h. In spite of the suppression of EGR1 expression, the invasive capacity was not affected in the EGR1 shRNA-transfected cells, compared to that in control shRNA-transfected cells (Figure 2b). Next, cell-migration was evaluated by comparing the effect of α-bisabolol between EGR1 shRNA-transfected cells and control shRNA-transfected cells. Although α-bisabolol suppressed cell motility in both cell lines, inhibition of EGR1 also had no influence on cell motility (Figure 2c). These data suggest that EGR1 has no relationship to the inhibitory effect of α-bisabolol on cellular invasiveness and motility.

Gene profiling concerning tumor metastasis in α-bisabolol-treated pancreatic cancer cell. To further investigate the key molecules that are related to the inhibitory effects of α-
Figure 1. α-Bisabolol suppressed the invasiveness and motility in pancreatic cancer cell lines. a: The invasiveness was assessed in α-bisabolol-treated pancreatic cancer cell lines KLM1 (left), KP4 (center), and Panc1 (right) using a Boyden chamber method. The cells were treated with 1.56 μM of α-bisabolol for 24 h. On each membrane, the invaded areas were measured at six randomly selected visual fields (×5 objective). Data represent the relative invasion rate compared to that of the untreated control. b: Cell motility was determined in α-bisabolol-treated pancreatic cancer cell lines KLM1 (left), KP4 (center), and Panc1 (right) using a cell migration assay. The cells were treated with 1.56 μM of α-bisabolol for 24 h. The distance traveled by the cells was measured at the same six points, and the mean distance was determined 0 and 24 h after treatment with α-bisabolol. The relative migration rate is shown relative to the migration distance in control cells. *p<0.05, **p<0.01.
Figure 2. Early growth response 1 (EGR1) has no influence on the inhibitory effect of α-bisabolol on invasiveness and motility. 

a: EGR1 expression was detected in wild-type KLM1, control shRNA-transfected KLM1 cells and EGR1 shRNA-transfected KLM1 cells after a 6-h treatment with 6.25 μM of α-bisabolol by western blot analysis. β-Actin was used as an internal control.

b: Cell invasiveness was assessed in control shRNA-transfected KLM1 cells and EGR1 shRNA-transfected KLM1 cells using Boyden chamber method. The cells were treated with 1.56 μM of α-bisabolol for 6 h. On each membrane, the invaded areas were measured at six randomly selected visual fields (×5 objective). Data represent the relative invasion rate compared to that of the control shRNA-transfected KLM1 cells. Cell invasiveness was not significantly altered by suppression of EGR1 expression.

c: Cell motility of EGR1 shRNA-transfected KLM1 cells and control shRNA-transfected KLM1 cells was assessed using a cell-migration assay. The distance traveled by the cells was measured at the same six points, and the mean distance was determined at 0, 12, 24, and 36 h after treatment with 1.56 μM α-bisabolol. The relative migration rate is shown relative to the migration distance in control shRNA-transfected KLM1 cells; **p<0.01. NS: Not significant.
bisabolol on invasiveness and motility of pancreatic cancer cells, PCR array analysis concerning tumor metastasis were performed on α-bisabolol-treated pancreatic cancer cell lines. Several genes, such as KISS1R, metastasis suppressor 1 (MTSS1), and tissue inhibitor of metalloproteinase 2 (TIMP2), were up-regulated in α-bisabolol-treated pancreatic cancer cells (Figure 3a). Among these genes, we focused on KISS1R. KISS1R and its ligand KISS1 are known as metastasis suppressors, inhibiting cancer cell migration and invasion (10). The data suggest that KISS1R might be one of the key molecules related to the inhibitory effect of α-bisabolol on cellular invasiveness and motility.

α-Bisabolol suppressed invasiveness through induction of KISS1R in pancreatic cancer cells. To examine whether KISS1R directly affects the inhibitory effect of α-bisabolol or not, invasiveness was analyzed in KISS1R-suppressed pancreatic cancer cell lines. Invasiveness was enhanced in KLM1 and KP4 cells upon treatment with KISS1R siRNA, but not sufficiently in Panc1 cells (Figure 4). The inhibitory effect of α-bisabolol was weakened in KISS1R-suppressed KLM1 and KP4 cells.

Discussion

We previously reported that α-bisabolol inhibited cell proliferation and induced apoptosis of pancreatic cancer in in vitro and in vivo experiments. Metastasis is defined as the secondary tumor at different organs from the primary tumor. Although complete surgical resection provides the only potential cure, the surgical outcome in pancreatic cancer remains unsatisfactory because of metastasis, peritoneal dissemination and local recurrence (10). Therefore, a novel treatment is urgently required to improve the prognosis of metastasis of pancreatic cancer. However, no studies on the efficiency of α-bisabolol on tumor invasiveness and motility, that are related to the metastatic potential of tumor cells, have been reported. This study first demonstrated that α-bisabolol suppressed the invasiveness and motility of a pancreatic cancer cell line.

Cavalieri et al. reported that α-bisabolol rapidly induced apoptosis of human malignant glioma cell lines through the mitochondrial pathway without toxicity to normal glial cells (7). In our previous report, we demonstrated that EGR1 was involved in the antiproliferative and apoptosis-inducing potentials of α-bisabolol in a pancreatic cancer cell line (9). In addition to our previous report, many reports demonstrated that EGR1 plays an important role in inhibiting cell-migration, proliferation, and angiogenesis (11-13). Therefore, we first investigated whether the induction of EGR1 by α-bisabolol had some impact on the invasiveness and motility of a pancreatic cancer cell line. Unexpectedly, EGR1 expression did not impact the inhibitory effects of tumor invasiveness and motility.
Figure 4. α-Bisabolol suppressed invasiveness through induction of Kisspeptin 1 receptor (KISS1R) in pancreatic cancer cells. Invasiveness was analyzed in KISS1R-suppressed pancreatic cancer cell lines. The cells were transfected with KISS1R siRNA or control siRNA, and then treated with 1.56 μM of α-bisabolol for 24 h. On each membrane, the invaded areas were measured at six randomly selected visual fields (×5 objective). Data represent the relative invasion rate compared to that of the untreated control siRNA-transfected cells; *p<0.05, **p<0.01. NS: Not significant.
KISS1R, also called G-protein coupled receptor 54 (GPR54), is a receptor for metastin (kisspeptin-54 or kp-54), which is a C-terminally amidated peptide of KISS1. Several reports showed that KISS1R suppressed the metastasis of various cancer types, such as melanoma, breast carcinoma, and gastrointestinal carcinoma by inhibiting cancer cell migration and invasion (14, 15). Masui et al. reported that KISS1/KISS1R signaling activated extracellular signal-regulated kinase (ERK1), but inhibited cell-migration in pancreatic cancer (16). Nagai et al. reported that high expression of KISS1 and KISS1R was associated with longer survival of patients with pancreatic cancer (17). KISS1R is also known as a metastasis-suppressor, inhibiting metastasis without affecting the growth rate of tumor (18, 19). Several genes, such as Kangai 1 (KAI1) and metastasis suppressor (MTSS), were identified as metastasis-suppressing genes in association with the progression of metastasis. Our data demonstrated that activation of KISS1R is a novel mechanism of α-bisabolol against pancreatic cancer. We consider that the inhibitory effects of α-bisabolol on tumor invasiveness and motility are, at least partly, associated with the activation of KISS1R. There is a possibility that other molecular mechanisms of α-bisabolol exist in regulating invasiveness and motility of pancreatic cancer cells.

In summary, our study demonstrated, as far as we are aware for the first time, that α-bisabolol can be a potent therapeutic drug against tumor invasiveness and motility in pancreatic cancer. These effects are associated with up-regulation of KISS1R. Further investigations are necessary to clarify the precise mechanisms of α-bisabolol action for clinical application as a novel treatment for pancreatic cancer.

Disclosure Statement

The Authors have no conflict of interest with regard to this study.

References