

Differential Anticancer Activity of Pterostilbene Against Three Subtypes of Human Breast Cancer Cells

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Abstract. Although pterostilbene, a natural analog of resveratrol, has potent antitumor activity against several human cancer types, the possible inhibitory mechanisms against subtypes of human breast cancer with different hormone receptor and human epidermal growth factor receptor 2 (HER2) status remain unknown. We investigated the anticancer activity of pterostilbene using three subtypes of breast cancer cell lines. Pterostilbene treatment exhibited a dose-dependent antiproliferative activity, with the greatest growth inhibition observed in triple-negative MDA-MB-468 cells. Although pterostilbene arrested cell-cycle progression at the G_0/G_1 phase regardless of breast cancer subtype, its apoptosis-inducing activity was highly apparent in MDA-MB-468 cells. Pterostilbene induced strong and sustained activation of extracellular signal-regulated kinase (ERK) 1/2, with concomitant cyclin D1 suppression and p21 up-regulation, and inhibited the phosphorylation of AKT and mammalian target of rapamycin (mTOR), followed by subsequent up-regulation of BAX without affecting B-cell lymphoma-extra large (BCL-xL). Oral administration of pterostilbene significantly suppressed tumor growth in nude mice xenotransplanted with MDA-MB-468 cells. These data suggest a potential role of pterostilbene for prevention and treatment of human breast cancer, especially of triple-negative breast cancer.

Breast cancer is the most frequently occurring cancer among women, and the leading cause of cancer death worldwide (1). Breast cancer is a heterogeneous disease that can be classified into several distinct subtypes based on estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth

factor receptor 2 (HER2) expression. Therapeutic options for patients with advanced breast cancer are limited and depend on the breast cancer subtype. Although survival of patients with breast cancer has been improved by hormonal and targeted therapy in recent years, triple-negative breast cancer, which is clinically negative for expression of ER, PR and HER2, is associated with a poor prognosis (2). Because there are a limited number of treatments for triple-negative breast cancer, development of novel treatment options and prevention for this type of breast cancer is extremely important. In this context, fruit and vegetables have attracted attention because their intake has been shown to reduce the risk of breast cancer (3), and differentially affect ER-negative and -positive breast cancer incidence rates, preferentially lowering the risk of ER-negative breast cancer in postmenopausal women (4). Furthermore, a recent cohort study showed that fruit intake in adolescence significantly reduces the risk of ER-negative, but not ER-positive premenopausal breast cancer (5). Such differential cancer-preventive potentials of fruit and vegetables are considered to be attributable to phytochemicals, since a growing number of studies have uncovered potential applications for phytochemicals for chemoprevention and treatment for cancer, not only in cell lines but also in animal models of cancer (6). Accordingly, we recently showed that phytochemicals such as lycopene and nobletin possess differential anticancer activity against breast cancer cells with different hormone receptor and HER2 status (7, 8).

Pterostilbene (*trans*-3, 5-dimethoxy-4'-hydroxystilbene) is a stilbenoid phytochemical with similar structure and biological activities to those of resveratrol (9). Like resveratrol, pterostilbene has multiple pharmacological properties, including antioxidant, anti-inflammatory, and cancer-preventive activity (10-12). Although many of the benefits associated with resveratrol also apply to pterostilbene, pterostilbene has a longer half-life and higher oral bioavailability (13). Moreover, it is more potent than resveratrol in inhibiting the growth of human colon cancer cells as well as preventing azoxymethane-induced colonic tumorigenesis (14). Although the excellent antioxidant property of pterostilbene is most likely the basis for its preventive role against cancer at the initiating and

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progression stages, increasing evidence indicates that pterostilbene has anticancer activity against a variety of types of human cancer cells through modulation of diverse molecular targets involving growth factor signaling, cell-cycle regulation, and cell survival/apoptosis (15-17). In animal studies, pterostilbene inhibited the growth of pancreatic and breast cancer in xenotransplanted nude mice (16, 18). These *in vitro* and *in vivo* studies suggest that pterostilbene might be potentially useful for the prevention and treatment of various types of human cancer. Since phytochemicals have been suggested to lower the risk of ER-negative rather than ER-positive breast cancer, it is of interest to determine whether there are any differences in pterostilbene activity among different subtypes of breast cancer cells. In the present study, we investigated the cellular and molecular mechanisms of the growth-inhibitory activity of pterostilbene against three human breast cancer cell lines that differ in their hormone receptor and HER2 status. In addition, an *in vivo* experiment using a mouse xenograft model was conducted to determine whether the inhibitory effects of pterostilbene are clinically applicable to human breast cancer.

Materials and Methods

Cell culture and chemicals. The study was performed on three subtypes of breast cancer cell lines, namely ER-positive MCF-7 cells, hormone receptor-negative but HER2-positive SK-BR-3 cells, and triple-negative MDA-MB-468 cells, all purchased from the American Type Culture Collection (Manassas, VA, USA). Origins of the cell lines and their hormone receptor and HER2 status have been described previously (19). These cell lines were cultured in RPMI-1640 (Wako, Osaka, Japan) supplemented with 10% fetal bovine serum (Life Technologies, Carlsbad, CA, USA), 100 IU/ml penicillin and 100 µg/ml streptomycin in a humidified atmosphere of 95% air and 5% CO₂ at 37°C. Pterostilbene was purchased from Carbosynth Ltd. (Berkshire, UK), and stored at -80°C. A 1.0 M solution was prepared by dissolving original pterostilbene with dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA). A 10 mM solution was prepared by diluting this 1.0 M pterostilbene solution with 99.5% ethanol (WAKO, Osaka, Japan) immediately before experimental use. The final concentration of DMSO for all experiments and treatments (including vehicle controls, where no drug was added) was maintained at 0.01% or less. These conditions were found to be non-cytotoxic for at least 72 h.

Determination of growth inhibition. The antiproliferative effects of pterostilbene on these breast cancer cell lines were assessed by Coulter counter (Beckman Coulter, Brea, CA, USA) rather than colorimetric WST-1 cell proliferation assay because we have found disagreement between the actual cell number as measured by Coulter counter and colorimetric assays due to the influence of pterostilbene on optical absorbance. Briefly, 2 ml cell suspension was seeded into 35mm dishes at density of 5×10⁴ cells per dish and incubated overnight. After addition of 0-100 µM pterostilbene, cells were further cultured for 72 h and the cell number was determined. The relative number of viable cells was determined by comparing the number of treated cells with the corresponding number of

vehicle-treated cells taken as 100%. Each experiment was performed using three replicate dishes for each pterostilbene concentration and was carried out independently three times. The IC₅₀ value defined as the concentration needed for a 50% reduction in cell viability, was determined. The antiproliferative effects of similar concentrations of resveratrol on these breast cancer cell lines were also assessed using the same method as described above.

Cell-cycle analysis and apoptosis measurement. At different times following treatment with 100 µM pterostilbene, floating and trypsinized adherent cells were combined, fixed in 70% ethanol, and stored at 4°C prior to cell-cycle analysis. We used this high concentration of pterostilbene, because lower concentration is insufficient to clearly visualize the difference of cell cycle distribution. After removal of ethanol by centrifugation, cells were washed with phosphate-buffered saline and stained with a solution containing RNase A and propidium iodide (Sigma-Aldrich). Cell-cycle analyses were performed on a Beckman Coulter Gallios Flow Cytometer using the Kaluza ver.1.2 software Packages (Beckman Coulter), and the extent of apoptosis was determined by measuring the sub-G₀/G₁ population.

Western blot analysis of signaling proteins for cell cycle, growth and apoptosis. Western blot analysis was performed as previously described (20). Equal amounts of proteins prepared by lysed cells treated with 100 µM pterostilbene were resolved by 4-15% sodium dodecylsulfate-polyacrylamide gel electrophoresis (Bio Rad, Hercules, CA, USA) and electrotransferred onto a polyvinylidene difluoride membrane (GE Healthcare, Piscataway, NJ, USA). Non-specific binding sites were blocked by incubating the membranes in blocking buffer (Nacalai Tesque, Kyoto, Japan) at room temperature for 30 min. The membranes were then incubated with primary antibodies against either phospho-mTOR (Ser2448) (Abcam, Cambridge, UK), phospho-p44/42 ERK1/2 (Thr202/Tyr204), phospho-AKT (Ser473), Cyclin D1 (2992), p21 (C19) (Santa Cruz Biotech, Dallas, TX, USA), BCL-xL (2762), or BAX (2772). The membranes were hybridized with horseradish peroxidase-conjugated secondary antibody. Immunoblots were then developed with the enhanced chemiluminescence system (GE Healthcare) and were then quantified using a LAS-3000 Luminescent Image Analyzer (Fuji Film, Tokyo, Japan). The blots were stripped and reprobed with primary antibodies against mTOR, ERK1/2, AKT and β-actin. All primary and secondary antibodies, except those for p21 and phospho-mTOR, were purchased from Cell Signaling Technology (Danvers, MA, USA). For reblotting, membranes were incubated in stripping buffer (Thermo, Rockford, IL, USA) for 30 min at room temperature before washing, blocking, and incubating with antibody. Triplicate determinations were made in separate experiments.

Animal experiments. Animal experiments were carried out in accordance with the protocols approved by the Institutional Animal Care and Use Committee of Nakamura Gakuen University (No. 2015-6). Two weeks prior to the inoculation of tumor cells, 12 female nude mice (BALB/cAJcl-nu/nu, CLEA Japan) at the age of 5 weeks were divided into two groups each consisting of six mice, and fed modified NIH-07 powder diet devoid of soy isoflavone (NIH-07PLD; Oriental Yeast Co. Ltd, Tokyo, Japan), supplemented with or without 0.1% pterostilbene (w/w) throughout the experiments. The composition of modified NIH-07PLD diet was described previously (21). Food intake and bodyweight were monitored throughout the duration of the experiment. Approximately 5×10⁶ MDA-MB-468 cells were injected

subcutaneously into the dorsal flank. The date of appearance and size of palpable tumors were monitored weekly over a period of 8 weeks. Tumors were measured using calipers, and tumor volume was calculated by the formula: tumor volume (mm³)=[length (mm)] × [width (mm)]² × 0.52.

Statistical analysis. To determine the significance of observed differences, analysis of variance (ANOVA) was applied to the data using SPSS statistical software (version 19 for Windows; IBM Corp., Armonk, NY, USA). Time-course analysis data of the cell cycle distribution was compared using Dunnett's test. The mean values of IC₅₀ were compared by Tukey's test. Comparisons of IC₅₀ between pterostilbene and resveratrol were made by Student's *t*-test. Data for bodyweight, food intake, and tumor volume in nude mice were analyzed by Student's *t*-test. Differences with a *p*-value less than 0.05 were considered significant.

Results

Effects of pterostilbene and resveratrol on proliferation and survival. Pterostilbene treatment for 72 consecutive hours led to dose-dependent growth inhibition of MCF-7, SK-BR-3, and MDA-MB-468 cells, with the greatest growth inhibition observed in MDA-MB-468 cells (Figure 1). The IC₅₀ value for MDA-MB-468 cells treated with pterostilbene for 72 h was significantly lower compared to that for MCF-7 cells, showing approximately 2-fold greater sensitivity to pterostilbene (Table I). HER2-positive SK-BR-3 cells were moderately sensitive to pterostilbene, with an IC₅₀ between those of triple-negative MDA-MB-468 and ER-positive MCF-7 cells. IC₅₀ values for resveratrol were similar to those of pterostilbene in the respective breast cancer cell lines, although pterostilbene was significantly more cytotoxic than resveratrol in SK-BR-3 cells (Table I).

Time-course analysis of the effect of pterostilbene on cell-cycle progression and apoptosis. To examine whether the inhibitory effects observed in cytotoxicity assays reflect the arrest or delay of cell-cycle progression or apoptotic cell death, cells were treated with pterostilbene at a dose of 100 μM, and cell-cycle progression and apoptosis were evaluated by fluorescence-activated cell sorting analysis. Treatment with pterostilbene significantly increased the population of G₀/G₁ phase cells from 62-64% at the beginning of the treatment to 70-72% after 24 h exposure, with a corresponding decrease in cells in G₂/M phase, irrespective of the subtype of breast cancer (Figure 2), and the percentages of cells in each cell-cycle phase did not significantly change afterwards (Figure 2). Although the percentage of the sub-G₀/G₁ cell population, which represents apoptotic cells, increased gradually with time in MCF-7 cells, the sub-G₀/G₁ cell population in SK-BR-3 cells significantly increased by approximately three-fold 72 h post-treatment (Figure 2b). By contrast, the sub-G₀/G₁ apoptotic cell population in MDA-MB-468 cells substantially increased after 24 h exposure (18.1%) and increased by nearly

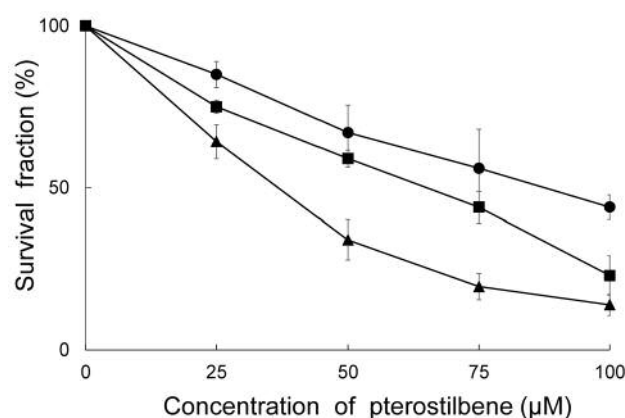


Figure 1. Survival curves for pterostilbene-treated MCF-7 (●), SK-BR-3 (■), and MDA-MB-468 (▲) cells. Cells were treated with different concentrations of pterostilbene for 72 h and the number of cells was measured by Coulter counter as described in the Materials and Methods. The data represent the means from three independent experiments. Bars=standard deviation (SD).

Table I. The concentrations of pterostilbene and resveratrol needed for a 50% reduction in cell viability (IC₅₀) in breast cancer cell lines treated for 72 h. Cells were treated with different concentrations of pterostilbene and resveratrol for 72 h. The IC₅₀ values were assessed by Coulter counter as described in the Materials and Methods section. Values are mean±SD of three independent experiments. IC₅₀ values for pterostilbene and resveratrol for individual cell lines were compared by Tukey's test: **p*<0.05, ***p*<0.01. Comparisons of IC₅₀ between pterostilbene and resveratrol were made by Student's *t*-test.

Cell line	IC ₅₀ (μM)		<i>p</i> -Value pterostilbene versus resveratrol
	Pterostilbene	Resveratrol	
MCF-7	87.6±9.0	88.8±6.7	0.863
SK-BR-3	64.4±4.6	75.3±3.1	0.026
MDA-MB-468	45.7±5.2	49.3±1.2	0.309

two-fold after 72 h exposure, resulting in 35.8% at which the greatest growth inhibition was observed (Figure 2c). These data indicate that pterostilbene-induced growth decline in SK-BR-3 and MDA-MB-468 cells appears to be mediated mainly by progressive expansion of the apoptotic cell population.

Effect of pterostilbene on activation of signaling molecules. Because activation of signaling molecules such as ERK1/2, AKT and mTOR have been considered as major factors contributing to proliferation and survival, we examined the effect of pterostilbene on expression or activation

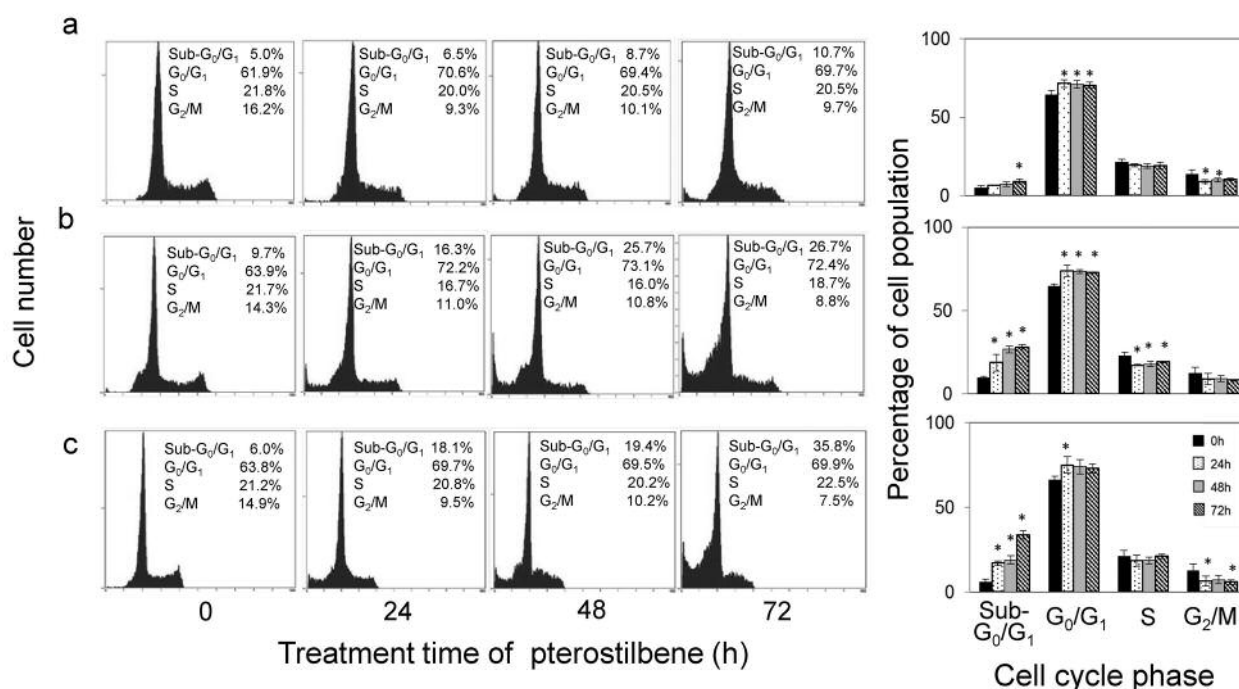


Figure 2. Time-course analysis of the effect of pterostilbene on cell-cycle progression and apoptosis as determined by flow cytometry. Representative cell-cycle distributions after exposure of MCF-7 (a), SK-BR-3 (b) and MDA-MB-468 (c) breast cancer cell lines to 100 μ M pterostilbene for 0, 24, 48 and 72 h. Percentages of the total cell population in the different phase of cell cycle are also shown in the right panels of each cell line. Data are the means \pm SD of three independent experiments. *Significant difference versus control (0 h) at $p < 0.05$.

(phosphorylation) of these proteins. Constitutive activity of ERK1/2 was enhanced 6 h after initiation of treatment with pterostilbene in SK-BR-3 and MDA-MB-468 cells, and remained at high levels over 24 h (Figure 3a). In contrast, the phosphorylation of AKT and its downstream molecule mTOR was inhibited time-dependently in MDA-MB-468 cells, and slightly inhibited in SK-BR-3 cells but not in MCF-7 cells (Figure 3a). Cyclin D1, functioning as a key regulator of the G₀/G₁ cell-cycle checkpoint, was inhibited with subsequent increase of p21 protein in all three subtypes of breast cancer cells, indicating that pterostilbene arrests the cell cycle at G₀/G₁ through a decrease in cyclin D1 expression and concomitant up-regulation of p21 regardless of breast cancer subtype.

Effects of pterostilbene on expression of pro- and anti-apoptotic proteins. To elucidate the apoptotic mechanism induced by pterostilbene, we examined the expression of anti-apoptotic BCL-xL as well as pro-apoptotic BAX (Figure 3b). Upon treatment with 100 μ M pterostilbene, BCL-xL expression did not change in any of the subtypes of breast cancer. By contrast, expression of BAX slightly increased with incubation time with pterostilbene in MCF-7, but was substantially enhanced

in SK-BR-3 and MDA-MB-468 cells. Therefore, enhanced expression of BAX appears to be responsible for pterostilbene-induced apoptotic induction in those breast cancer cell lines. A slight increase in BAX expression in MCF-7 cells might explain the lower apoptosis induction in these cells compared to SK-BR-3 and MDA-MB-468 cells.

Effects of pterostilbene on MDA-MB-468 cells xenotransplanted into nude mice. MDA-MB-468 triple-negative cells were found to be most sensitive to pterostilbene. Therefore, we conducted an *in vivo* experiment using nude mice xenotransplanted with MDA-MB-468 cells to determine whether the inhibitory effects of pterostilbene were clinically applicable to human breast cancer. There were no significant differences of weight gain and consumption of food between mice fed the control diet and those fed pterostilbene-containing diet over 10 weeks (Table II), suggesting no signs of toxicity induced by pterostilbene. Although tumors grew rapidly in mice fed the control diet, addition of pterostilbene apparently suppressed tumor growth over 10 weeks (Figure 4), reducing the tumor size by 76% compared to the control diet (Table II). These data indicate that pterostilbene substantially inhibits tumor growth *in vivo* without apparent toxicity.

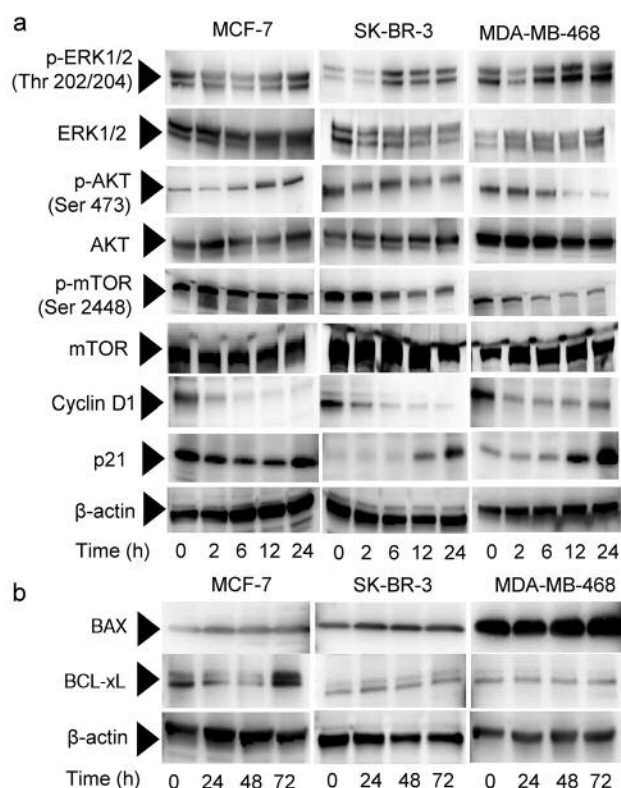


Figure 3. Effects of pterostilbene on activation of signaling molecules for cell proliferation and survival. Cells were treated with 100 μ M pterostilbene for the indicated times and harvested for western blot analysis. (a) Western blots are shown for phosphorylated and total ERK1/2, AKT, and mTOR. Cyclin D1 and p21 are also shown. (b) Effects of pterostilbene on apoptotic and anti-apoptotic proteins BAX and BCL-xL. β -Actin was used as an internal control.

Discussion

In the present study, we found that pterostilbene has differential anticancer activity against three subtypes of human breast cancer that differ in their hormone receptor and HER2 status. Pterostilbene exhibited a dose-dependent growth-inhibitory activity, with the greatest inhibition observed in triple-negative MDA-MB-468 cells. ER-positive MCF-7 cells were the least sensitive to pterostilbene, while HER2-positive SK-BR-3 cells were moderately sensitive. Compared to resveratrol, the IC_{50} values for pterostilbene were similar to those of resveratrol in the three respective subtypes of breast cancer cells, indicating that pterostilbene and resveratrol exhibit almost equivalent anticancer activity. In contrast to our data, a previous report showed pterostilbene to be more potent than resveratrol in inhibiting the growth of human colon cancer cells (14). Therefore, the anticancer activity of these resveratrol derivatives may vary depending on the cancer cell origin. Because of its longer

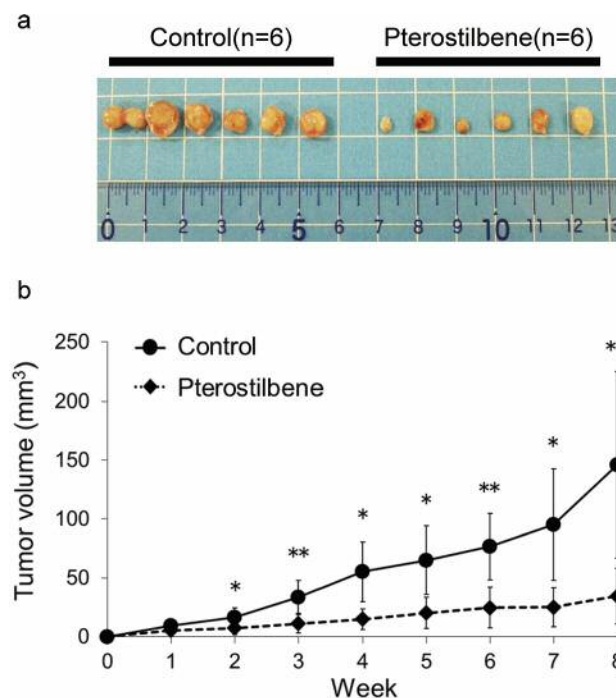


Figure 4. Effects of pterostilbene on tumor from MDA-MB-468 cells xenotransplanted into nude mice. The experimental treatment protocol is described in the Materials and Methods section. a: Tumors excised from six individual mice in each group are shown. b: Time course changes of the mean tumor volume in control mice and pterostilbene-treated mice implanted with MDA-MB-468 cells. Six samples were analyzed in each group, and values represent the mean \pm SD. Significantly different at * p <0.05, and ** p <0.01.

Table II. Bodyweight, tumor volume, and total food consumption of mice xenotransplanted with MDA-MB-468 cells.

	Control (n=6)	Pterostilbene (n=6)	<i>p</i> -Value ^a
Bodyweight (g) ^b	25.1 \pm 1.3	25.3 \pm 1.9	0.625
Tumor volume (mm ³) ^b	146.0 \pm 79.3	34.6 \pm 23.7	0.008
Food intake (g) ^c	311.0 \pm 15.2	327.8 \pm 19.3	0.305

Data are presented as the mean \pm SD. ^aStudent's *t*-test, control versus pterostilbene. ^bDetermined at age of 15 weeks. ^cTotal food consumption during experiment.

half-life and higher oral bioavailability than resveratrol (13), pterostilbene would be superior as a potential preventive phytochemical for breast cancer overall and, more specifically, ER-negative breast cancer such as HER2-positive and triple-negative breast cancer. Moreover, using

nude mice xenotransplanted with triple-negative MDA-MB-468 cells, pterostilbene substantially inhibited tumor growth without apparent toxicity, suggesting a potential clinical application in humans. Correspondingly, pterostilbene has been shown to be generally safe for use in humans up to 250 mg/day (22). Taken together, these data suggest the therapeutic usefulness of pterostilbene in the treatment of human breast cancer, thus warranting clinical evaluation of pterostilbene in patients with triple-negative breast cancer.

In the present study, pterostilbene induced a substantial delay of the cell cycle at G₀/G₁ phase regardless of breast cancer subtype, and reduced the expression of cyclin D1, with a concomitant increase of p21 protein. Previous studies reported that pterostilbene induced G₀/G₁ cell-cycle arrest, corroborated by the down-regulation of cyclin D1 with/without up-regulation of p21 in several human cancer cell lines including human prostate (23), gastric (17), and hormone-dependent mammary cancer (24) cells. Our results, together with the previous findings, suggest that the arrest of the cell cycle at G₀/G₁ through down-regulation of cyclin D1 and/or up-regulation of p21 might be a universal event triggered by pterostilbene treatment. The molecular mechanism whereby pterostilbene arrests cells in the G₀/G₁ phase remains unclear. However, enhancement of constitutive activity of ERK1/2 may cause pterostilbene-induced G₀/G₁ block as demonstrated in breast cancer cells treated with lycopene (8). ERK1/2 is an important subfamily of mitogen-activated protein kinases that controls a broad range of cellular activities and physiological processes (25). Activation of ERK1/2 generally has proliferative capacity, but under certain conditions, ERK1/2 has been shown to have antiproliferative activity through G₁ cell-cycle arrest (26). A robust early phase of ERK1/2 signaling followed by a moderate sustained phase leads to transient induction of p21 and accumulation of cyclin D1, allowing G₁ progression. However, robust and prolonged activation of ERK1/2 causes G₁ arrest due to long-term p21 induction and cyclin-dependent kinase 2 inhibition (26). In the present study, continuous treatment with pterostilbene enhanced the constitutive activity of ERK1/2 from 6 h, which remained at high levels with p21 induction over 24 h in SK-BR-3 and MDA-MB-468 cells. Therefore, pterostilbene-induced strong and sustained activation of ERK pathway may cause G₀/G₁ cell cycle blockade in breast cancer cells.

We found that the growth-inhibitory activity of pterostilbene is mediated by differential mechanisms of action, depending on ER and HER2 status. Pterostilbene substantially induced apoptotic cell death by increasing the expression of BAX, without affecting the BCL-xL level in SK-BR-3 and MDA-MB-468 cells. In contrast, ER-positive MCF-7 cells, which were found to be twofold more resistant to pterostilbene than triple-negative MDA-MB-468 cells, showed marginal induction of apoptosis, with slight

upregulation of BAX. It has been suggested that AKT plays a critical role in controlling survival and apoptosis through direct phosphorylation of mTOR, thereby inducing signals leading to anti-apoptotic pathway. In this study, pterostilbene clearly inactivated AKT and downstream mTOR in triple-negative MDA-MB-468 cells, but not in ER-positive cells. Therefore, these data suggest that pterostilbene may execute its apoptotic effect primarily through blocking AKT/mTOR-mediated signaling pathways in triple-negative cells. Similar pterostilbene-induced apoptosis through suppression of AKT signaling pathway has been reported in breast cancer and colon cancer cell lines (16, 27). Furthermore, pterostilbene also induces apoptosis in human oral cancer cell lines by reducing phosphorylation of AKT and mTOR (28). Taken together, pterostilbene induces apoptosis by enhancing the expression of BAX through inactivation of AKT and mTOR, indicating the important role of BAX in pterostilbene-induced apoptosis induction. Although additional research is needed to identify the mechanisms accounting for the lower sensitivity of ER-positive cells to pterostilbene, our data may support the epidemiological evidence that fruit and vegetable intake reduces the risk of ER-negative rather than ER-positive breast cancer (4, 5).

Despite recent advances in breast cancer treatment, breast cancer recurrence is a major problem and represents the principal cause of breast cancer-related death. Emerging evidence suggests the existence of cancer stem cells, a population of cells capable of self-renew and initiating tumor growth, which might be responsible for breast cancer recurrence (29). Recently, phytochemicals have gained immense attention because of their wide safety profile and ability to target heterogeneous populations of cancer cells, including cancer stem cells (30). Moreover, pterostilbene has been reported to selectively kill breast cancer stem cells isolated from MCF-7 cells (31). In our study, pterostilbene inhibited the growth of xenotransplanted triple-negative MDA-MB-468 tumors consisting of a population of *in vivo*-selected and thus highly tumorigenic cells resembling cancer stem cells. In this regard, our data suggest that pterostilbene might be potentially useful not only for prevention and treatment, but also the reduction of recurrence of human breast cancer, especially triple-negative breast cancer.

Conflicts of Interest

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

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References

- Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A: Global cancer statistics, 2012. *CA Cancer J Clin* 65(2): 87-108, 2015.
- Cetin I and Topcul M: Triple-negative breast cancer. *Asian Pac J Cancer Prev* 15(6): 2427-2431, 2014.
- Aune D, Chan DS, Vieira AR, Rosenblatt DA, Vieira R, Greenwood DC and Norat T: Fruits, vegetables and breast cancer risk: A systematic review and meta-analysis of prospective studies. *Breast Cancer Res Treat* 134(2): 479-493, 2012.
- Fung TT, Hu FB, McCullough ML, Newby PK, Willett WC and Holmes MD: Diet quality is associated with the risk of estrogen receptor-negative breast cancer in postmenopausal women. *J Nutr* 136(2): 466-472, 2006.
- Farvid MS, Chen WY, Michels KB, Cho E, Willett WC and Eliassen AH: Fruit and vegetable consumption in adolescence and early adulthood and risk of breast cancer: Population based cohort study. *BMJ* 353: i2343, 2016.
- Bathaie SZ, Faridi N, Nasimian A, Heidarzadeh H and Tamanoi F: How phytochemicals prevent chemical carcinogens and/or suppress tumor growth. *Enzymes* 37: 1-42, 2015.
- Chen C, Ono M, Takeshima M and Nakano S: Antiproliferative and apoptosis-inducing activity of nobiletin against three subtypes of human breast cancer cell lines. *Anticancer Res* 34(4): 1785-1792, 2014.
- Takeshima M, Ono M, Higuchi T, Chen C, Hara T and Nakano S: Anti-proliferative and apoptosis-inducing activity of lycopene against three subtypes of human breast cancer cell lines. *Cancer Sci* 105(3): 252-257, 2014.
- Estrela JM, Ortega A, Mena S, Rodriguez ML and Asensi M: Pterostilbene: Biomedical applications. *Crit Rev Clin Lab Sci* 50(3): 65-78, 2013.
- McCormack D and McFadden D: A review of pterostilbene antioxidant activity and disease modification. *Oxid Med Cell Longev* 2013: 575482, 2013.
- Paul S, Rimando AM, Lee HJ, Ji Y, Reddy BS and Suh N: Anti-inflammatory action of pterostilbene is mediated through the p38 mitogen-activated protein kinase pathway in colon cancer cells. *Cancer Prev Res* 2(7): 650-657, 2009.
- Rimando AM, Cuendet M, Desmarchelier C, Mehta RG, Pezzuto JM and Duke SO: Cancer chemopreventive and antioxidant activities of pterostilbene, a naturally occurring analogue of resveratrol. *J Agric Food Chem* 50(12): 3453-3457, 2002.
- Kapetanovic IM, Muzzio M, Huang Z, Thompson TN and McCormick DL: Pharmacokinetics, oral bioavailability, and metabolic profile of resveratrol and its dimethylether analog, pterostilbene, in rats. *Cancer Chemother Pharmacol* 68(3): 593-601, 2011.
- Chiou YS, Tsai ML, Nagabhushanam K, Wang YJ, Wu CH, Ho CT and Pan MH: Pterostilbene is more potent than resveratrol in preventing azoxymethane (AOM)-induced colon tumorigenesis via activation of the NF-E2-related factor 2 (NRF2)-mediated antioxidant signaling pathway. *J Agric Food Chem* 59(6): 2725-2733, 2011.
- Mannal PW, Alosi JA, Schneider JG, McDonald DE and McFadden DW: Pterostilbene inhibits pancreatic cancer *in vitro*. *J Gastrointest Surg* 14(5): 873-879, 2010.
- Pan C, Hu Y, Li J, Wang Z, Huang J, Zhang S and Ding L: Estrogen receptor- α 36 is involved in pterostilbene-induced apoptosis and anti-proliferation in *in vitro* and *in vivo* breast cancer. *PLoS One* 9(8): e104459, 2014.
- Pan MH, Chang YH, Badmaev V, Nagabhushanam K and Ho CT: Pterostilbene induces apoptosis and cell-cycle arrest in human gastric carcinoma cells. *J Agric Food Chem* 55(19): 7777-7785, 2007.
- McCormack DE, Mannal P, McDonald D, Tighe S, Hanson J and McFadden D: Genomic analysis of pterostilbene predicts its antiproliferative effects against pancreatic cancer *in vitro* and *in vivo*. *J Gastrointest Surg* 16(6): 1136-1143, 2012.
- Neve RM, Chin K, Fridlyand J, Yeh J, Baehner FL, Fevr T, Clark L, Bayani N, Coppe JP, Tong F, Speed T, Spellman PT, DeVries S, Lapuk A, Wang NJ, Kuo WL, Stilwell JL, Pinkel D, Albertson DG, Waldman FM, McCormick F, Dickson RB, Johnson MD, Lippman M, Ethier S, Gazdar A and Gray JW: A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. *Cancer Cell* 10(6): 515-527, 2006.
- Ono M, Higuchi T, Takeshima M, Chen C and Nakano S: Differential antitumor activities of curcumin against RAS- and SRC-activated human adenocarcinoma cells. *Biochem Biophys Res Commun* 436(2): 186-191, 2013.
- Ono M, Koga T, Ueo H and Nakano S: Effects of dietary genistein on hormone-dependent rat mammary carcinogenesis induced by ethyl methanesulphonate. *Nutr Cancer* 64(8): 1204-1210, 2012.
- Riche DM, McEwen CL, Riche KD, Sherman JJ, Wofford MR, Deschamps D and Griswold M: Analysis of safety from a human clinical trial with pterostilbene. *J Toxicol* 2013: 463595, 2013.
- Lin VC, Tsai YC, Lin JN, Fan LL, Pan MH, Ho CT, Wu JY and Way TD: Activation of ampk by pterostilbene suppresses lipogenesis and cell-cycle progression in p53- positive and -negative human prostate cancer cells. *J Agric Food Chem* 60(25): 6399-6407, 2012.
- Wang Y, Ding L, Wang X, Zhang J, Han W, Feng L, Sun J, Jin H and Wang XJ: Pterostilbene simultaneously induces apoptosis, cell-cycle arrest and cytoprotective autophagy in breast cancer cells. *Am J Transl Res* 4(1): 44-51, 2012.
- Brown MD and Sacks DB: Protein scaffolds in map kinase signalling. *Cell Signal* 21(4): 462-469, 2009.
- Meloche S and Pouyssegur J: The ERK1/2 mitogen-activated protein kinase pathway as a master regulator of the G1- to S-phase transition. *Oncogene* 26(22): 3227-3239, 2007.
- Tolba MF and Abdel-Rahman SZ: Pterostilbene, an active component of blueberries, sensitizes colon cancer cells to 5-fluorouracil cytotoxicity. *Sci Rep* 5: 15239, 2015.
- Ko CP, Lin CW, Chen MK, Yang SF, Chiou HL and Hsieh MJ: Pterostilbene induce autophagy on human oral cancer cells through modulation of AKT and mitogen-activated protein kinase pathway. *Oral Oncol* 51(6): 593-601, 2015.
- Ahmad A: Pathways to breast cancer recurrence. *ISRN Oncol* 2013: 290568, 2013.
- Dandawate PR, Subramaniam D, Jensen RA and Anant S: Targeting cancer stem cells and signaling pathways by phytochemicals: Novel approach for breast cancer therapy. *Semin Cancer Biol* 40-41: 192-208, 2016.
- Wu CH, Hong BH, Ho CT and Yen GC: Targeting cancer stem cells in breast cancer: Potential anticancer properties of 6-shogaol and pterostilbene. *J Agric Food Chem* 63(9): 2432-2441, 2015.

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