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Urokinase-type plasminogen activator expression and Rac1/WAVE-2/Arp2/3 pathway are blocked by pterostilbene to suppress cell migration and invasion in MDA-MB-231 cells ☆

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ABSTRACT

Breast cancer is the most common malignancy among females, and cancer invasion and metastasis are the leading causes of cancer death in breast cancer patients. Pterostilbene, a naturally occurring dimethylether analogue of resveratrol, has been demonstrated to possess anti-cancer effects. However, inhibitory effects of pterostilbene on cell migration and invasion and its underlying mechanisms are not fully understood. In this study, we investigated the anti-invasive mechanisms of pterostilbene in human breast cancer cell line MDA-MB-231 cells. Pterostilbene effectively inhibited serum-induced migration and invasion without affecting the viability of breast cancer cells. The mRNA expression and activity of urokinase-type plasminogen activator (uPA) were markedly reduced by pterostilbene treatment. Moreover, pterostilbene attenuated nuclear factor κB (NF-κB) transcriptional activity and DNA binding of NF-κB on uPA promoter. In addition, pterostilbene significantly impaired the activity of Rac1 and the expression of WASP-family verprolin-homologous protein-2 (WAVE-2) and actin-related protein 2/3 (Arp2/3). Overall, these results suggest that pterostilbene caused considerable suppression of cell migration and invasion through blocking NF-κB-mediated uPA expression and Rac1/WAVE/Arp2/3 pathway.

Breast cancer is the most common malignancy among females,[1] and frequently metastasizes to bone, lymph node, brain and lung,[2] which interrupts successful treatment of breast cancer. Metastasis still considers as the significant cause of mortality being responsible for 90% of cancer deaths.[2,3]

It is well characterized that urokinase-type plasminogen activator (uPA) system, which is a serine protease family and consists of uPA, uPA receptor (uPAR) and plasminogen activator inhibitors, plays a crucial role in breast cancer metastasis. The binding of uPA to uPAR facilitates extracellular matrix (ECM) degradation, angiogenesis, adhesion, migration and invasion.[4–6] Duffy et al.[7] first identified the connection between uPA activity in primary breast cancer and both tumor size and metastatic status, and demonstrated that breast cancer patients with high uPA activity had a poor disease-free interval than those with low activity. Thus, uPA system may be a prognostic marker for aggressive breast cancer and an ideal candidate target for cancer therapy.

Rac, a member of Rho family small GTPases, plays a vital role in controlling cell motility. Rac activates actin-related protein 2/3 (Arp2/3) complex via WASP-family verprolin-homologous proteins (WAVEs), thereby inducing reorganization of actin cytoskeleton at the leading edge and consequent formation of lamellipodia.[8,9] Regulation of these processes is important for cancer therapy because actin cytoskeleton reorganization is the primary mechanism of cell motility and is dynamically occurred during cell migration.[8] Zhang et al. reported that (−)-Epigallocatechin-3-gallate inhibited breast cancer cell migration and invasion by attenuating Rac1 activity.[10] Furthermore, recent study has demonstrated that Cucurbitacin E decreased tumor cell migration by impairing Arp2/3-dependent actin polymerization in breast cancer.[11]

Pterostilbene is a naturally occurring dimethylether analogue of resveratrol and stilbene phytoalexin found in several types of berries and grapes.[12–14] Pterostilbene is reported to have the beneficial effects such as anti-inflammatory, anti-oxidant, analgesic, anti-diabetic, and hypolipidemic activities.[14–16] In addition, pterostilbene has been suggested to exhibit anti-cancer potentials including inhibition of cell proliferation and induction of apoptosis in many different types of human cancers as effective as resveratrol due to their close similarity in structure.[12,17–19] Few studies demonstrated that pterostilbene had the ability to suppress cancer cell invasion and metastasis in vitro and in vivo.[17,20–22] However,

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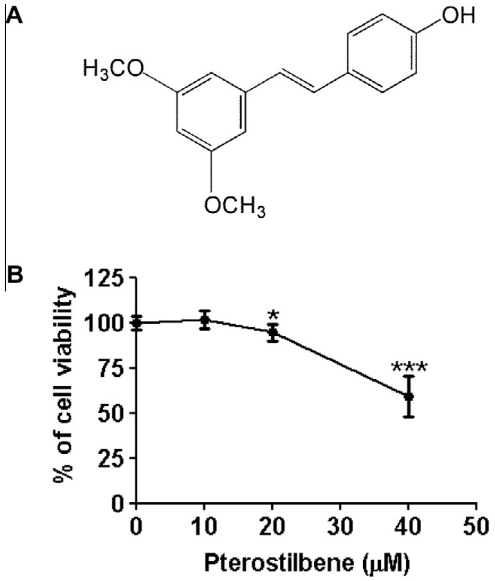

Figure 1. Cytotoxic effect of pterostilbene against MDA-MB-231 cells. Cells were treated with the indicated concentrations of pterostilbene for 24 h, followed by assessment for cell viability by the MTT assay. Data are shown as means ± SD of three independent experiments by analysis of Student's *t* test. $^{*}p < 0.05$ and $^{***}p < 0.001$ versus untreated control.

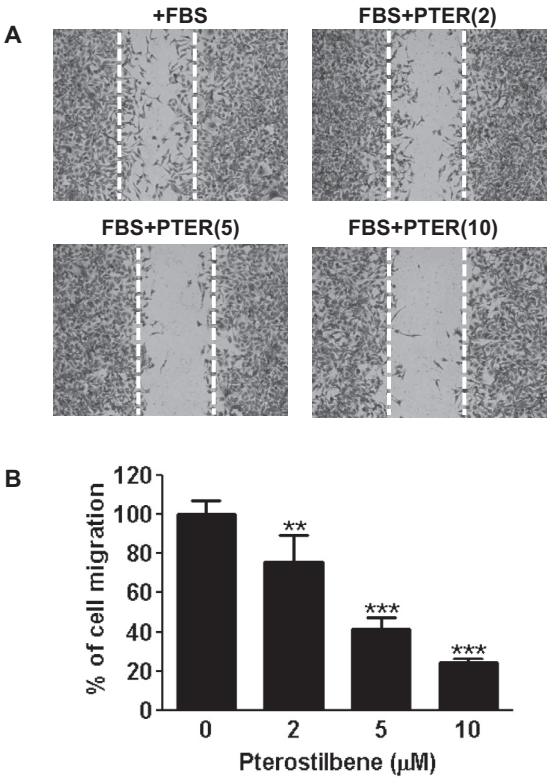

Figure 2. Pterostilbene inhibited the migration of MDA-MB-231 cells. The migration ability of cells was carried out by the wound healing assay. The confluent cells were scratched and then treated with pterostilbene in a serum-containing culture medium for 24 h. (A) The cell were stained with Diff-Quick and then randomly chosen fields were photographed at 100×. (B) The number of cells migrated into the scratched area was calculated as a percentage of migration. Data are shown as means ± SD of two independent experiments by analysis of Student's *t* test. $^{**}p < 0.01$ and $^{***}p < 0.001$ versus serum-treated control.

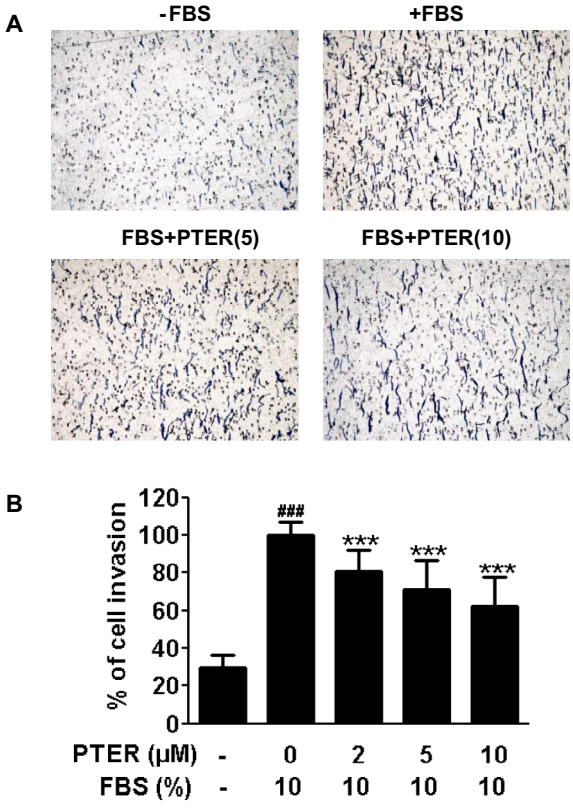

Figure 3. Pterostilbene inhibited the invasion of MDA-MB-231 cells. The invasion assay was performed using 48-well microchemotaxis chambers with matrigel-coated filter for 15 h. (A) The cell were stained with Diff-Quick and then randomly chosen fields were photographed at 100×. (B) The number of cells invaded to the lower surface was calculated as a percentage of invasion. Data are shown as means ± SD of three independent experiments by analysis of Student's *t* test. $^{###}p < 0.001$ versus untreated control and $^{***}p < 0.001$ versus serum-treated control.

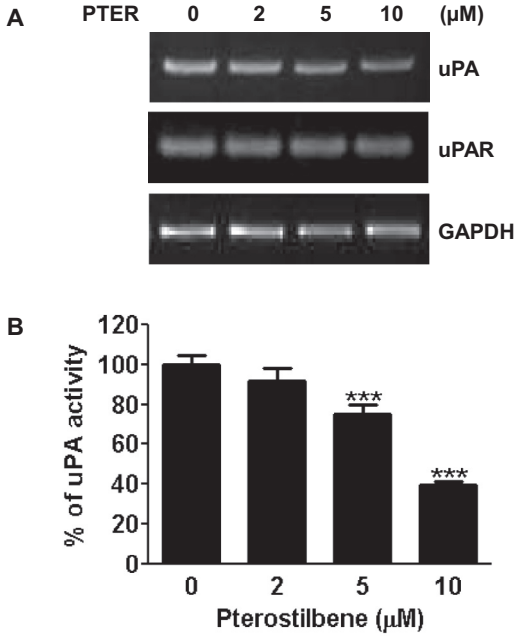

Figure 4. Pterostilbene reduced the mRNA expression and activity of uPA in MDA-MB-231 cells. Cells were treated with pterostilbene for 24 h. (A) The mRNA expression of uPA and uPAR were determined by RT-PCR analysis. GAPDH was used as a loading control. (B) The activity of uPA was assessed using a SPECTROZYME® PL in conditioned medium from pterostilbene-treated MDA-MB-231 cells. Data are shown as means ± SD of two independent experiments by analysis of Student's *t* test. $^{***}p < 0.001$ versus untreated control.