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Effect of β-Phenylethyl Isothiocyanate from Cruciferous Vegetables on Growth Inhibition and Apoptosis of Cervical Cancer Cells through the Induction of Death Receptors 4 and 5

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ABSTRACT: Cruciferous vegetables have been shown to have the possibility to protect against multistep carcinogenesis. β-Phenylethyl isothiocyanate (PEITC) is one component of these vegetables demonstrated to help fight many types of cancer. The present study examined the apoptotic effects of PEITC and its molecular mechanism in human cervical cancer cell lines (HEp-2 and KB). PEITC induced apoptosis to inhibit cell proliferation. According to the protein chip assay, PEITC increased the expression of the death receptors (DR4 and DR5) and cleaved caspase-3 compared to the DMSO treatment group. PEITC also induced caspase-8 and truncated BID. PEITC down-regulated the phosphorylation of extracellular-related kinase (ERK)1/2, whereas neither phospho-c-Jun NH_2-terminal kinases (JNK) nor phospho-p38 MAPK was changed. The role of ERK in PEITC-induced apoptosis was also investigated using MEK inhibitor (PD98059). PD98059 increased the expression of DR4 and DR5, activated caspase-3, and cleaved PARP. In addition, PEITC decreased the phosphorylation of MEK. Therefore, the apoptotic mechanism of PEITC in cervical cancer cells involves the induction of DR4 and DR5 through the inactivation of ERK and MEK.

KEYWORDS: PEITC, cervical cancer cells, death receptors, ERK1/2, MEK

INTRODUCTION

Cervical cancer is the second most common leading cause of cancer-related death in women worldwide with an approximate incidence of 450,000 newly diagnosed cases each year with a mortality rate of 50%.[1−4] Cervical cancer occurs in women in most developing countries, where it may comprise approximately one-fourth of female cancers. Therefore, finding new strategies to limit cervical cancer has been the goal of many studies.

Dietary fruits and vegetables are considered potential sources of chemopreventive compounds and have been investigated widely owing to their low toxicity but significant chemopreventive efficacies.[5] Epidemiological studies continue to justify the hypothesis that dietary intake of cruciferous vegetables may help reduce the risk of many malignancies.[6,7] The anticarcinogenic effect of cruciferous vegetables may be attributed to organic isothiocyanates (ITCs) in a variety of edible cruciferous vegetables including broccoli, watercress, and cabbage.[6] Many ITCs are effective chemopreventive agents against carcinogen-induced cancers in experimental animals.[8−12] PEITC is characterized by phenolic and sulfur-containing N═C═S functional groups and is one of the ITC family of compounds that exhibit cancer chemopreventive activity.[10] Recent studies have demonstrated the effect of PEITC as well as other ITC analogues on suppressing cell proliferation of cancer cells by causing apoptosis and/or G_2/M phase cell cycle arrest in human leukemia, hepatoma, and prostate cancer cells.[13−22] However, there are no reports of the effect of PEITC and its molecular mechanism on cervical carcinogenesis.

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a member of the TNF superfamily, induces the apoptosis of tumor cells of different origins but not normal cells in vitro or in normal cells of experimental animals, thereby providing therapeutic possibilities in human cancers.[23−25] The significant importance of the TRAIL pathway in cancer is highlighted by the TRAIL receptor. TRAIL can bind to two death receptors (DRs), DR4 (TRAIL-R1) and DR5 (TRAIL-R2), that contain a cytoplasmic functional death domain. Death receptors DR4 and DR5 are type I transmembrane proteins containing an intracellular death domain that engages the apoptotic machinery upon TRAIL binding.[26] The level of DR4 and/or DR5 expression plays a decisive role in determining the intensity and/or duration of TRAIL-induced death receptor-mediated apoptotic signaling. There are at least two fundamental apoptotic pathways,

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which are referred to as the extrinsic and intrinsic pathways. TRAIL can trigger either pathway, depending on the cell type.[27] TRAIL-induced apoptosis initiated by the extrinsic pathway involves the following: DR engagement, death-inducing signaling complex (DISC) formation, proteolytic activation of caspase-8, and, consequently, activation of caspase-3.[28] This highlights the role of the death receptors related to the extrinsic pathway in inducing apoptosis in human cancer. Many studies have clarified the potential role of the up-regulation of death receptors induced by naturally occurring chemicals in producing apoptosis.[29−32] Nevertheless, there are no reports of the effect of PEITC in regulating death receptors that eventually assist in positive chemotherapy against cervical cancer cells.

In the present study, we investigated the effect of PEITC on the growth of cancer cells and its signaling pathway in human cervical cancer cell lines (HEp-2 and KB). This study provides evidence that the cell death caused by PEITC is initiated by the down-regulation of the MEK/ERK signaling pathway, resulting in an increase in DR4 and DR5 and leading to apoptosis in human cervical cancer cells.

MATERIALS AND METHODS

Reagents and Antibodies. PEITC (C_9H_9NS) and 4′-6-diamidino-2-phenylindole (DAPI) were obtained from Sigma-Aldrich Inc. (St. Louis, MO). 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) with a CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay Kit was supplied by Promega Corp. (Madison, WI). MEK inhibitor (PD98059) was provided by Calbiochem Technology (San Diego, CA). Antibodies recognizing cleaved caspase-3(Asp175), cleaved caspase-8, DR5, BID, phosphorylated ERK at threonine 202/tyrosine 204 and total ERK, phosphorylated JNK at threonine 183/tyrosine 185 and total JNK, phosphorylated p38 at threonine 180/tyrosine 182 and total p38, and phosphorylated MEK at serine 217/221 were purchased from Cell Signaling Technology (Denver, MA). Antibodies against HO-1, total MEK, and actin were acquired from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). DR4 antibody was obtained from R&D Systems Inc. (Minneapolis, MN), and PARP antibody was supplied by BD Bioscience (San Diego, CA).

Cell Culture and Drug treatment. HEp-2 cells were obtained from Kyungpook National University (Daegu, Korea), and KB cells were obtained from American Type Culture Collection (Manassas, VA). HEp-2 cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 100 U/mL each of penicillin and streptomycin; KB cells were cultured in DMEM supplemented with 5% FBS and 100 U/mL each of penicillin and streptomycin in a humidified atmosphere containing 5% CO_2 at 37 °C. Equal numbers of cells were seeded and allowed to attach. When the cells reached 50−60% confluence, the cells were treated with DMSO or PEITC (2.5, 5, and 10 μM) diluted in DMEM with 5% FBS for HEp-2 cells and 2.5% FBS for KB cells. PEITC was dissolved in 0.1% DMSO (vehicle control).

MTS Assay. The MTS assay was carried out using a CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay Kit to examine the effect of PEITC on the growth of HEp-2 and KB cells according to the manufacturer's instructions. After PEITC treatment for 24 and 48 h, a MTS solution was added to each well (20 μL for HEp-2 cells; 10 μL for KB cells) and incubated at 37 °C. The absorbance was measured at 490 nm using an ELISA reader (BIO-TEK Instruments, Inc., Madison, WI). The data were expressed as the percentage of cell viability compared to the control.

Western Blot Analysis. HEp-2 and KB cells were grown in 60 mm dishes and treated with DMSO or PEITC for the indicated time points

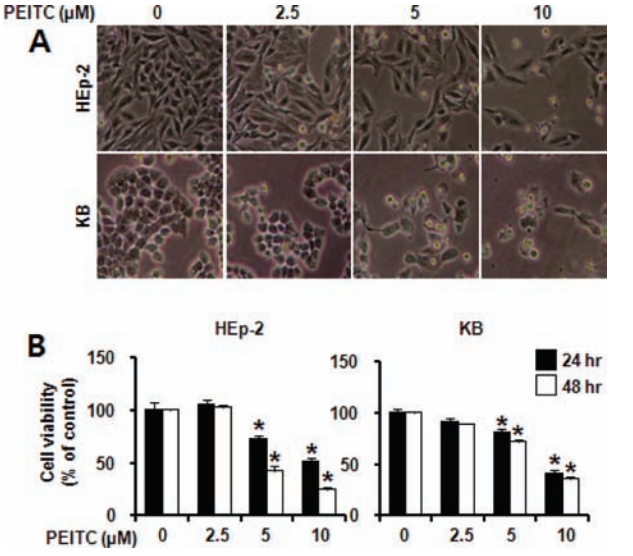

Figure 1. Cell growth inhibition caused by PEITC in human cervical cancer HEp-2 and KB cells. Cells were treated with DMSO (vehicle control) or various concentrations of PEITC (0, 2.5, 5, and 10 μM) for 24 and 48 h. (A) Cell viability was determined by cell photo taken by optical microscope after 48 h (magnification ×200). (B) MTS assay was performed to evaluate cell viability. The graphs indicate the mean ± SD of three independent experiments. ∗, $p < 0.05$ compared to the DMSO treatment group.

required for each experiment. Whole-cell lysates were extracted and quantified using a protein assay kit. Total protein from each sample was mixed with 5× loading buffer and heated at >90 °C for 5 min. Equal amounts of protein were separated by SDS−polyacrylamide gel electrophoresis (PAGE) and transferred onto poly(vinylidene fluoride) (PVDF) membrane (Bio-Rad Laboratories). The membranes were then blocked with 5% skim milk in TBST buffer at room temperature for 2 h, washed with TBST, and incubated overnight at 4 °C with primary antibody. Washing was done with TBST, and incubation with horseradish peroxidase (HRP)-conjugated secondary antibody was done at room temperature for 2 h. After washing with TBST, detection was performed with ECL Western blotting reagents (Santa Cruz, CA). For loading control, the membranes were stripped and reprobed with HRP-conjugated anti-β-actin.

DAPI Staining. Apoptotic cell death was determined morphologically using a fluorescent nuclear dye, DAPI. This showed the number of apoptotic cells with chromatin condensation and nuclear fragmentation. HEp-2 and KB cells were incubated with DMSO or PEITC (5, 10 μM) for 48 h, then harvested by trypsinization, and fixed in 70% ethanol overnight at −4 °C. The next day, the cells were stained with DAPI, deposited onto the slides, and finally viewed to detect apoptotic characteristics with a fluorescent microscope.

Protein Chip Assay (Apoptosis Chip). HEp-2 cells were seeded in 60 mm dishes and treated with DMSO (vehicle control) or 5 μM PEITC for 48 h and then harvested for protein chip assay using a Proteome Profiler Array-Human Apoptosis Array Kit (R&D Systems, Inc.) following the manufacturer's instructions. Briefly, cell lysates were extracted, then added to the arrays blocked by array buffer 1 for 1 h at room temperature, and incubated overnight at 4 °C. After washing with washing buffer, the arrays were exposed with Human Apoptosis Detection Antibody Cocktail for 1 h at room temperature. The cells were then washed and incubated with streptavidin−HRP for 30 min at room temperature. Finally, the arrays were washed and used to analyze the apoptosis-related proteins of PEITC on HEp-2 cells using chemiluminescent reagents.

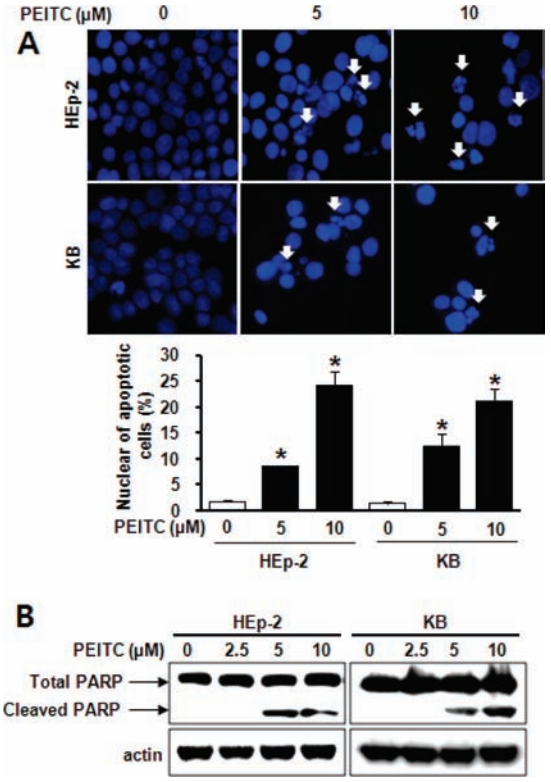

Figure 2. Apoptotic effects induced by PEITC in HEp-2 and KB cells. Cells were incubated with DMSO (vehicle control) or PEITC (5 and 10 μM) for 48 h. Then, cells were harvested and prepared for DAPI staining and Western blotting as described under Materials and Methods. (A) DNA fragmentation and nuclear condensation (white arrows) were detected by fluorescence microscopy (magnification ×400). DNA fragmentation and nuclear condensation were quantified, and the results in triplicates are expressed as the mean ± SD. *, $p < 0.05$ compared to the DMSO treatment group. (B) Apoptotic marker protein, cleaved PARP in whole cell lysates, was detected by Western blotting, and actin was used as loading control.

Statistical Analysis. The data were assessed for statistical significance using a Student's *t* test. A value of $p < 0.05$ compared to the vehicle control was considered to be statistically significant.

RESULTS

PEITC Decreases the Cell Number and Cell Viability of HEp-2 and KB Cells. To investigate the growth-inhibitory effect of PEITC in cervical cancer, HEp-2 and KB cells were treated with DMSO or various concentrations of PEITC. The result showed that the number of cells decreased and cells were detached in a concentration-dependent manner (Figure 1A). The effect of PEITC on cell viability was examined using a colorimetric MTS assay. The result showed a significant decrease in cell viability by PEITC (Figure 1B). This shows that PEITC is a potent growth inhibitor of cervical cancer cells.

Induction of Apoptosis by PEITC in HEp-2 and KB Cells. To investigate whether PEITC-induced growth inhibition was related to apoptotic effect, apoptosis induction upon the treatment of HEp-2 and KB cells with PEITC was performed using DAPI staining and Western blotting for cleaved PARP, which is a characteristic feature of cells undergoing apoptosis. Representative microscopic images of DAPI-stained cells following a 48 h exposure to DMSO (vehicle control) or 5 and 10 μM PEITC for three independent experiments are shown in Figure 2A. The results revealed the condensed and fragmented nuclei in the PEITC-treated group, but no changes in the DMSO treatment group. In addition, PARP cleavage was detected in both cell lines (Figure 2B). Overall, these findings suggested that PEITC could induce apoptosis in both cells.

Apoptotic Effect Caused by PEITC through the Up-regulation of Death Receptors (DR4, DR5) in HEp-2 Cell Line. Because we found that PEITC induced apoptosis to inhibit cancer cell growth, we wondered what kinds of signal molecules are involved in PEITC-induced apoptosis. Therefore, a protein chip assay was carried out to compare the changes in expression of certain signaling proteins related to apoptotic cell death between PEITC (5 μM)-treated and DMSO-treated groups-(Figure 3A). The results showed 4 proteins (DR4, DR5, cleaved caspase-3,and HO-1) of 35 proteins were increased significantly after the PEITC treatment compared to the DMSO treatment group (Figure 3B). Furthermore, the protein chip assay was confirmed by Western blotting. As can be seen in Figure 3C, PEITC caused a concentration-dependent increase in protein level of death receptors (DR4, DR5), cleaved caspase-3, and HO-1 in HEp-2 cells. Thus, this suggests that PEITC can increase DR4 and DR5, caspase-3, and HO-1 to induce apoptotic cell death.

PEITC Induces the Extrinsic Apoptotic Pathway and Is Caspase-Dependent in HEp-2 Cells. Because DR4 and DR5 are involved in PEITC-induced apoptosis, indicating that it is an extrinsic pathway, caspase-8 and BID were detected. The result in Figure 4A reveals that cleaved caspase-8 and cleaved BID were concentration-dependently increased by PEITC in HEp-2 cells. To confirm the involvement of caspase-8 in PEITC-induced apoptosis, caspase-8 inhibitor was used. The results showed that caspase-8 inhibitor partially blocked PEITC-induced apoptosis (Figure 4B). The previous study showed that PEITC-induced apoptosis in human prostate cancer cell lines was significantly attenuated in the presence of caspase inhibitor.[20] We therefore tested the caspase dependence of PEITC-induced apoptosis in HEp-2 cells. We treated HEp-2 cells with PEITC at 10 μM concentration after pretreatment with zVAD-fmk (pancaspase inhibitor) for 1 h. The cleaved PARP and cleaved caspase-3 induced by PEITC were clearly blocked in the presence of zVAD-fmk (Figure 4C). Consequently, this clearly indicated that apoptotic induction of PEITC in HEp-2 cells was mediated by caspases.

PEITC Decreased Phosphorylation of ERK1/2 but Did Not Cause Any Changes in JNK and p38 Levels. To further investigate if PEITC-induced apoptosis through death receptors is associated with MAPK pathway, the phosphorylations of ERK1/2, JNK, and p38 were detected. PEITC interrupted the activation of ERK1/2 through down-regulation of ERK1/2 phosphorylation in both cell lines. The treatment of 10 μM PEITC in KB and HEp-2 cells for 24 h reduced phospho-ERK protein levels to 0.307- and 0.042-fold compared to the control (Figure 5A, upper lane), whereas the total ERK remained unchanged regardless of the concentrations and time points (Figure 5A, lower lane). On the other hand, PEITC did not cause any changes in the phosphorylation or the total expression of JNK and p38 (Figure 5B). These data indicate that ERK1/2 can play an important role in PEITC-regulated apoptosis.

MEK Inhibitor, PD98059, Affects Cell Viability, Apoptosis, and DR4 and DR5. To further confirm whether the inactivation

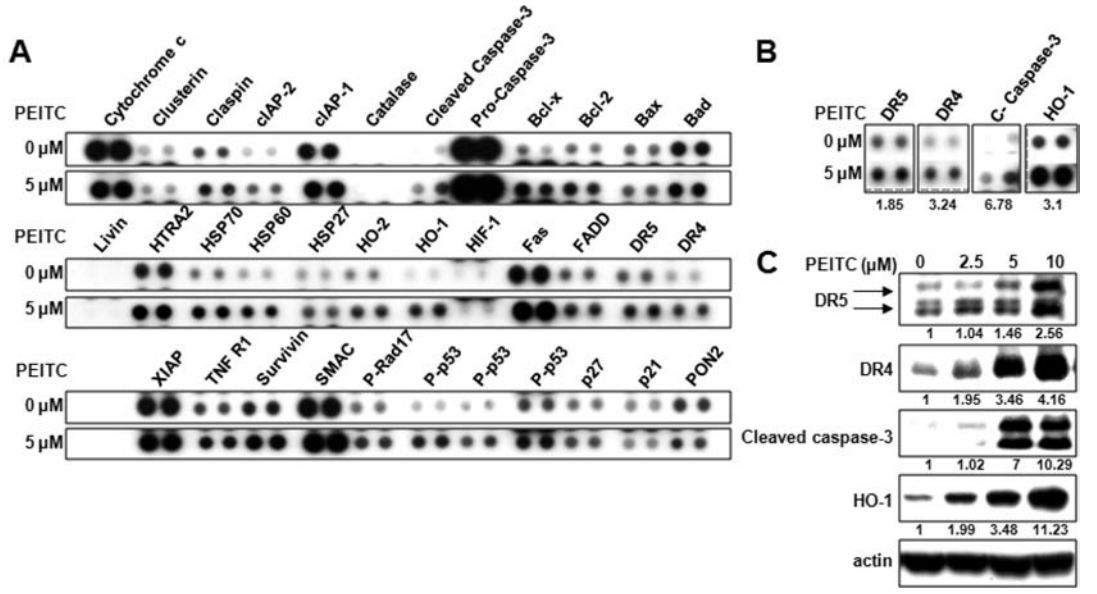

Figure 3. Induction of death receptors (DRs) by PEITC in HEp-2 cells. HEp-2 cells were treated with DMSO or 5 μM PEITC for 48 h and then harvested and analyzed by protein chip assay as described under Materials and Methods. (A) The PEITC-treated group indicated changes in expression of some proteins compared with the DMSO treatment group. (B) The result from protein chip focused on death receptors (DR4, DR5), cleaved caspase-3, HO-1 proteins with increase in protein level after PEITC treatment compared with the DMSO treatment (the data were calculated by Image-J program) in duplicates. (C) Confirmation of the results obtained by protein chip assay in HEp-2 cells in triplicates.

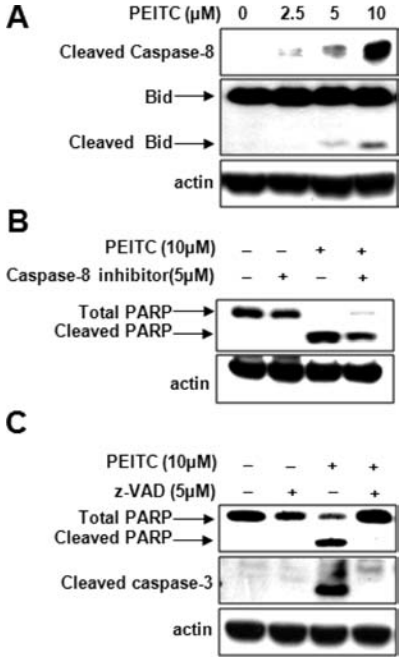

Figure 4. (A) Cleaved caspase-8 and cleaved BID as well as total BID were detected using Western blot in HEp-2 cells exposed to different concentrations of PEITC for 48 h. HEp-2 cells were treated with PEITC after 1 h of pretreatment of caspase-8 inhibitor (B) or pancaspase inhibitor (zVAD-fmk) (C) and then used for Western blotting to detect PARP and caspase-3.

of ERK1/2 was required for apoptotic cell death in human cervical cancer cells and induction of DR4 and DR5 in HEp-2 and KB cells, the MEK inhibitor, PD98059, was used. The cells were treated with PD98059 at 40 μM concentration in HEp-2 cells and at 80 μM concentration in KB cells for 48 h, and then its effects on cell viability and apoptosis were observed using the colorimetric MTS assay and Western blotting. It caused a significant growth inhibition (>40%) in both cell lines (Figure 6A). We also found that PD98059 increased the expression of death receptors (DR4, DR5), activated caspase-3, and cleaved PARP in HEp-2 and KB cells (Figure 6B). These results suggest that ERK1/2 is associated with PEITC-induced DR4 and DR5 to inhibit cell growth and induce apoptosis in human cervical cancer cells.

PEITC Also Caused Down-regulation of the Upstream Target of ERK1/2. We next verified the influence of PEITC treatment on MEK, the upstream kinase of ERK1/2. We found that the phosphorylation of MEK was concentration-dependently decreased in HEp-2 cells (Figure 7A, upper lane) as well as in KB cells (Figure 7B, upper lane). However, the total MEK remained unchanged (Figure 7, lower lanes). The data suggest that PEITC inactivates the MEK/ERK signaling pathway, leading to apoptosis.

DISCUSSION

Recent studies have reported that a high consumption of vegetables, particularly cruciferous vegetables, is associated with a reduced risk of many cancers.[13−21] Dietary fruits and vegetables are considered to be rich sources of chemopreventive compounds and have been widely investigated due to their low toxicity but significant chemopreventive efficacy.[5] PEITC is a putative chemopreventive compound from cruciferous vegetables with low toxicity. In the present study, we showed that PEITC can inhibit cell growth and induce apoptosis in human cervical cancer cells as evidenced by the decreased cell viability, nucleus condensation, DNA fragmentation, and the cleavage of PARP. Other studies reported that the marked concentrations contributing to the successful anticarcinogenetic activity of PEITC

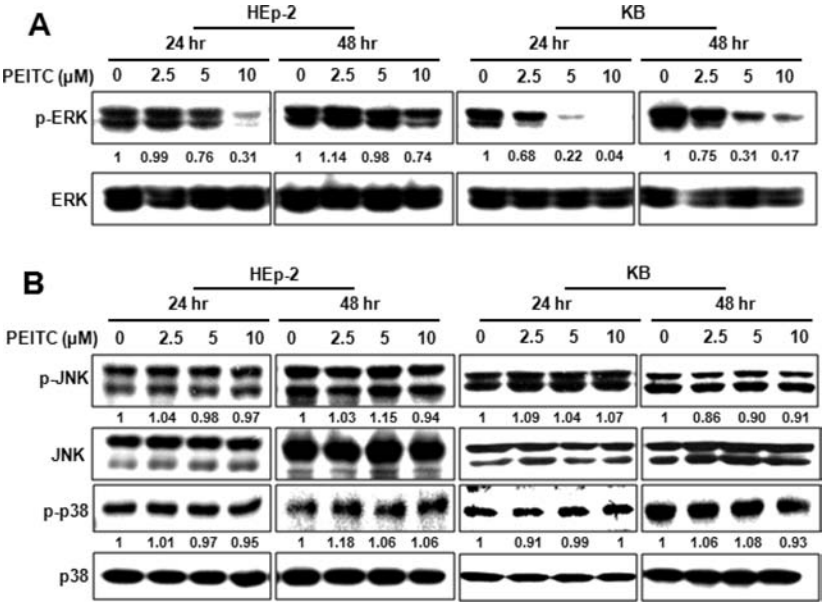

Figure 5. PEITC-induced apoptosis was associated with ERK1/2 pathway. HEp-2 and KB cells were treated with various concentrations of PEITC at two time points (24 and 48 h) to investigate the effect of PEITC on phospho-ERK1/2 and ERK1/2 (A) or phospho-JNK (p-JNK), phosphor-p38 (p-p38), JNK, and p38 (B) using Western blotting in triplicates.

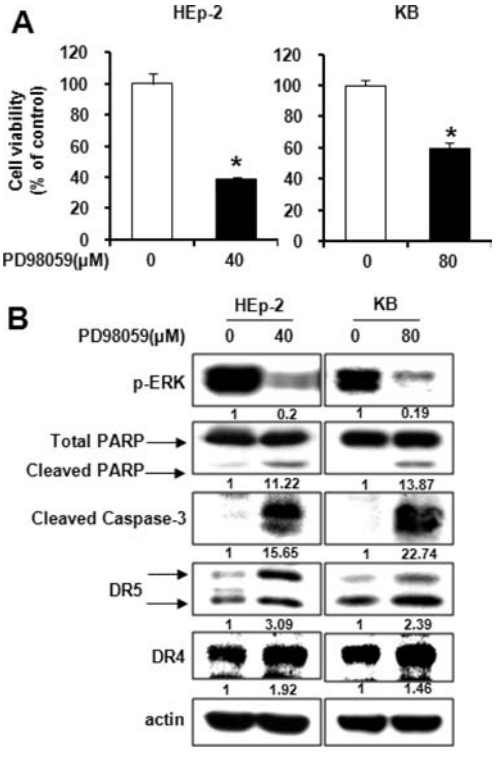

Figure 6. Effect of MEK inhibitor on cell viability, apoptosis, and death receptors. Cells were treated with DMSO (vehicle control) or MEK inhibitor (PD98059) at 40 μM in HEp-2 cells and at 80 μM in KB cells for 48 h. (A) Cell viability was detected using MTS assay. The graphs indicate the mean ± SD of three independent experiments. *, $p < 0.05$ compared to the DMSO treatment group. (B) ERK1/2, phospho-ERK1/2, DR4, DR5, caspase-3, and PARP were detected by Western blotting in triplicates.

against different types of cancer ranged from 2.5 to 10 μM, similar to the concentrations used in this study.[18,20,33−35]

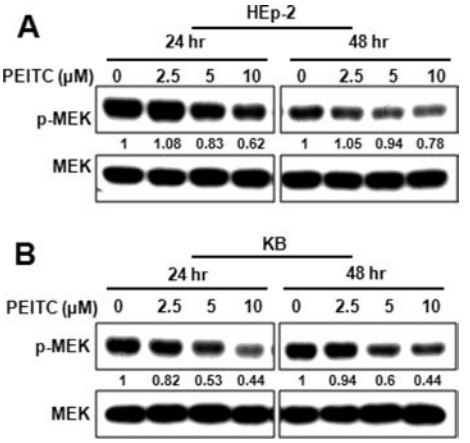

Figure 7. PEITC also down-regulated the upstream target of ERK (MEK). HEp-2 and KB cells were treated with various concentrations of PEITC at two time points (24 and 48 h). Phospho-MEK and total MEK were detected in HEp-2 (A) and KB (B) cells in triplicates.

The types of signal transduction proteins involved in PEITC-induced apoptosis were examined because PEITC can be a good compound to induce apoptosis in human cervical cancer cells. Therefore, a protein chip assay for apoptosis was performed. The results showed cleaved caspase-3, DR4, DR5, and HO-1 were significantly increased after PEITC treatment compared to the control followed by the confirmation using Western blotting. Caspases belong to a family of cysteine proteases that play a key role in apoptosis machinery. These enzymes participate in a cascade that is triggered in response to pro-apoptotic signals and culminates in the cleavage of a set of target proteins, with the specific cleavage site after aspartic acid, resulting in disassembly of the cell.[36] Caspases were shown to be induced by PEITC in HeLa and HL-60 cells, suggesting that caspases play important roles in PEITC-induced apoptosis.[37,38] In the present study, we

demonstrated that PEITC-induced apoptosis was completely blocked by a pancaspase inhibitor (zVAD-fmk), indicating that caspases are involved in the induction of apoptosis by PEITC. Natural products have played a significant role in the discovery of dietary compounds with anticancer activity.[39] Although no study has indicated the direct involvement of PEITC in the expression of the DRs, it has been reported that certain dietary compounds up-regulate the DRs and enhance apoptosis. In some studies, this activity was attributed to the inhibition of cell proliferation and apoptosis. Sulforaphane, a chemical of a family of compounds similar to ITCs, was shown to increase the DRs that eventually regulate apoptosis, metastasis, and angiogenesis.[40] Other chemicals, such as capsaicin, curcumin, esculetin, BB-1, and nimbolide, which are derived from natural plants, are also known to induce apoptosis through an increase in DRs.[29−32,41] Similarly, it was hypothesized that PEITC originating from cruciferous vegetables would have the same effect on the expression of the DRs. In the present study, PEITC clearly induced the expression of DR4 and DR5 proteins, which is in agreement with other studies. Because DR4 and DR5 are associated with the treatment of PEITC in human cervical cancer cells, the apoptotic pathway of PEITC could be extrinsic. Thus, we investigated whether caspase-8 and BID are affected by PEITC. The results revealed the dramatic cleavage of caspase-8 and BID. In addition, caspase-8 inhibitor partially blocked PEITC-induced apoptosis. These results indicate that PEITC induces apoptosis through the extrinsic pathway via the involvement of caspase-8 in part. Although capase-8 is involved in PEITC-induced apoptosis, the results also suggested that other pathways such as an apoptosome- or mitochondria-dependent pathway could not be ruled out.

MAPKs are serine/threonine superfamily kinases consisting of ERK1/2, JNK, and p38 MAPK that are involved in a range of cellular programs including cell growth, cell differentiation, cell development, inflammation, and apoptosis.[42] Modulation of the MEK/ERK pathway is associated with certain cellular responses, such as regulation of the cell cycle, apoptosis, proliferation, intercellular communication via gap junction, and signaling molecules depending on cell type, strength, and duration of the signal.[36,43,44] The activation of ERK was observed in PEITC-mediated apoptosis.[18,20,33,35] On the other hand, PEITC is also known to inactivate the ERK level, leading to apoptosis.[7,34] Therefore, the role of ERK in PEITC-induced apoptosis is still controversial, so we investigated whether PEITC affects the MAPK pathway. The results showed the concentration- and time-dependent suppression of ERK1/2 as indicated by its decreased phosphorylation by PEITC, whereas the two other members of the MAPK family known as JNK and p38 were not changed, suggesting that the inactivation of ERK1/2 by PEITC results in the induction of apoptosis. Because PEITC induced apoptosis and increased the levels of DRs, we raised the question of whether the down-regulation of ERK1/2 by PEITC resulted in the up-regulation of death receptors leading to apoptosis. Thus, we sought to confirm the effect of MEK inhibitor (PD98059) on cell viability, apoptosis, and the expression levels of DR4 and DR5. Our results showed that the dephosphorylation ability of PD98059 was justified by the down-regulation of phospho-ERK1/2 and that cell viability was markedly reduced in the presence of PD98059. In addition, it resulted in the activation of caspase-3 and the cleavage of PARP, which are the hallmarks of apoptotic activity as well as the up-regulation of DR4 and DR5. These results confirmed that DR4 and DR5 were strongly associated with ERK1/2 for PEITC-induced apoptosis in human cervical cancer cells. Moreover, MEK is the MAP kinase known to activate ERK1/2. A similar experiment indicated that MEK level was attenuated by PEITC, suggesting that PEITC regulates apoptotic cell death and DR4 and DR5 protein in human cervical cancer cells through suppression of MEK/ERK1/2 activity but not JNK or p38 MAPK.

The chemopreventive properties of ITCs have been attributed to the suppression of carcinogen bioactivation by cytochrome P450 isozymes (phase I) and increased detoxification and elimination of carcinogens via the induction of enzymes (phase II) associated with its antioxidant activity. PEITC is also considered to be a chemopreventive agent because of its induction of phase II enzymes both in vitro and in vivo.[45−47] Recently, PEITC treatment strongly increased the expression of antioxidant enzyme HO-1,[35] which appears to have anticancer effects. The data from the protein chip also showed that HO-1 is clearly induced by PEITC in a concentration-dependent manner. Therefore, future studies will investigate the molecular mechanism underlying the antioxidant effect of PEITC in human cervical cancer through the induction of HO-1.

In summary, PEITC has pro-apoptotic and antiproliferative effects in human cervical cancer cells. It also provides experimental evidence that the cell death caused by PEITC is initiated by the down-regulation of MEK/ERK signaling pathway to induce DR4 and DR5, leading to apoptosis in human cervical cancer cells. Therefore, we suggest that PEITC may be a promising dietary compound for cervical cancer by targeting DR4 and DR5.

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Author Contributions

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ABBREVIATIONS USED

PEITC, β-phenylethyl isothiocyanate; ITCs, isothiocyanates; DMSO, dimethyl sulfoxide; MAPK, mitogen-activated protein kinase; ERK, extracellular-related kinase; JNK, c-Jun NH_2-terminal kinase; MEK, MAPK/ERK kinase; TRAIL, TNF (tumor necrosis factor)-related apoptosis-inducing ligand; DR(s), death receptor(s); DR4, death receptor 4/TNF-related apoptosis-inducing ligand receptor 1; DR5, death receptor 5/TNF-related apoptosis-inducing ligand receptor 2; HO-1, heme oxygenase 1; PARP, poly ADP ribose polymerase; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; ECL, enhanced chemiluminescence.

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