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Phenethyl isothiocyanate sensitizes glioma cells to TRAIL-induced apoptosis

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ABSTRACT

Tumor necrosis factor-related apoptosis-induced ligand (TRAIL) is a promising antitumor therapy. However, many cancer cells, including malignant glioma cells, tend to be resistant to TRAIL, highlighting the need for strategies to overcome TRAIL resistance. Here we show that in combination with phenethyl isothiocyanate (PEITC), exposure to TRAIL induced apoptosis in TRAIL-resistant glioma cells. Subtoxic concentrations of PEITC significantly potentiated TRAIL-induced cytotoxicity and apoptosis in glioma cells. PEITC dramatically upregulated DR5 receptor expression but had no effects on DR4 receptor. PEITC enhances TRAIL-induced apoptosis through the downregulation of cell survival proteins and the upregulation of DR5 receptors through actions on the ROS-induced-p53.

1. Introduction

Glioblastoma multiforme (GBM; WHO grade IV) is the most common and most aggressive type of primary brain tumor with a high mortality rate. The median survival time of patients with GBM is less than 1 year even with multimodality therapy consisting of a combination of surgery, radiation and chemotherapy [1]. Therefore, finding new strategies to limit brain tumor has been the goal widespread research.

Many studies have founded that potential chemotherapeutic agent's increase TRAIL death receptor expression, increasing cytotoxicity they have founded that in different types of cancer cells [2,3]. In addition, cell death increase is caused by a synergistic apoptotic response from combination with TRAIL [3–5]. TRAIL binds to death receptors-4 and -5 (DR-4 and DR-5). This leads to the activation of caspase 8 and subsequent cleavage of BID, a BH3-only family member, causing mitochondrial dysfunction and apoptosis, the process of programmed cell death (PCD) [6]. TRAIL induces apoptosis in cancer cells without inducing apoptosis in normal cells. This indicates the potential for TRAIL to become a therapeutic agent in the treatment of cancer. Unfortunately, many cancer cells have resistance to TRAIL [7,8] and novel agents are needed to overcome this resistance to improve TRAIL efficacy. Hence, understanding the regulation of TRAIL receptor activation could provide insight into increasing TRAIL-induced apoptosis in these TRAIL-resistant cells. Thus, we and others are currently seeking to identify TRAIL sensitizers capable of overcoming TRAIL resistance in cancer cells [9,10].

Phenethyl isothiocyanate (PEITC) is one of the best studied members of isothiocyanates (ITC), a variety of edible cruciferous vegetables including broccoli, watercress, and cabbage, and have generated particular interest because of its remarkable chemopreventive activity.

In this study, we show that combined treatment with subtoxic doses of PEITC and TRAIL dramatically induces apoptosis in TRAIL-resistant glioma cells. The sensitizing effect of PEITC on TRAIL is associated with ROS generation and up-regulation of p53 protein levels. Our data suggests that both extrinsic and intrinsic pathways are involved in apoptosis induced by combined treatment with PEITC and TRAIL.

Abbreviations: PEITC, Phenethyl isothiocyanate; ROS, reactive oxygen species; NAC, N-acetyl-cysteine; PAGE, polyacrylamide gel electrophoresis; PARP, poly (ADP-ribose) polymerase; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand.

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2. Materials and methods

2.1. Reagents and antibodies

PEITC was purchased from LKT Laboratories (St. Paul, MN, USA). Soluble recombinant TRAIL was purchased from R&D Systems (Plymouth Meeting, PA, USA). The caspase inhibitors zDEVD-fmk (caspase-3 inhibitor), zLEHD-fmk (caspase-9 inhibitor) and zIETD-fmk (caspase-8 inhibitor) were purchased from Sigma–Aldrich (St. Louis, MO, USA). These caspase inhibitors were prepared and dissolved in dimethylsulfoxide (DMSO) and applied to the cells at 25 μM. 6-carboxy-2′,7′-dichlorofluorescein diacetate (H2DCF-DA) and MitoSOX were from Molecular Probe. Anti-caspase-3 antibody and anti-p53 antibody were purchased from Santa Cruz

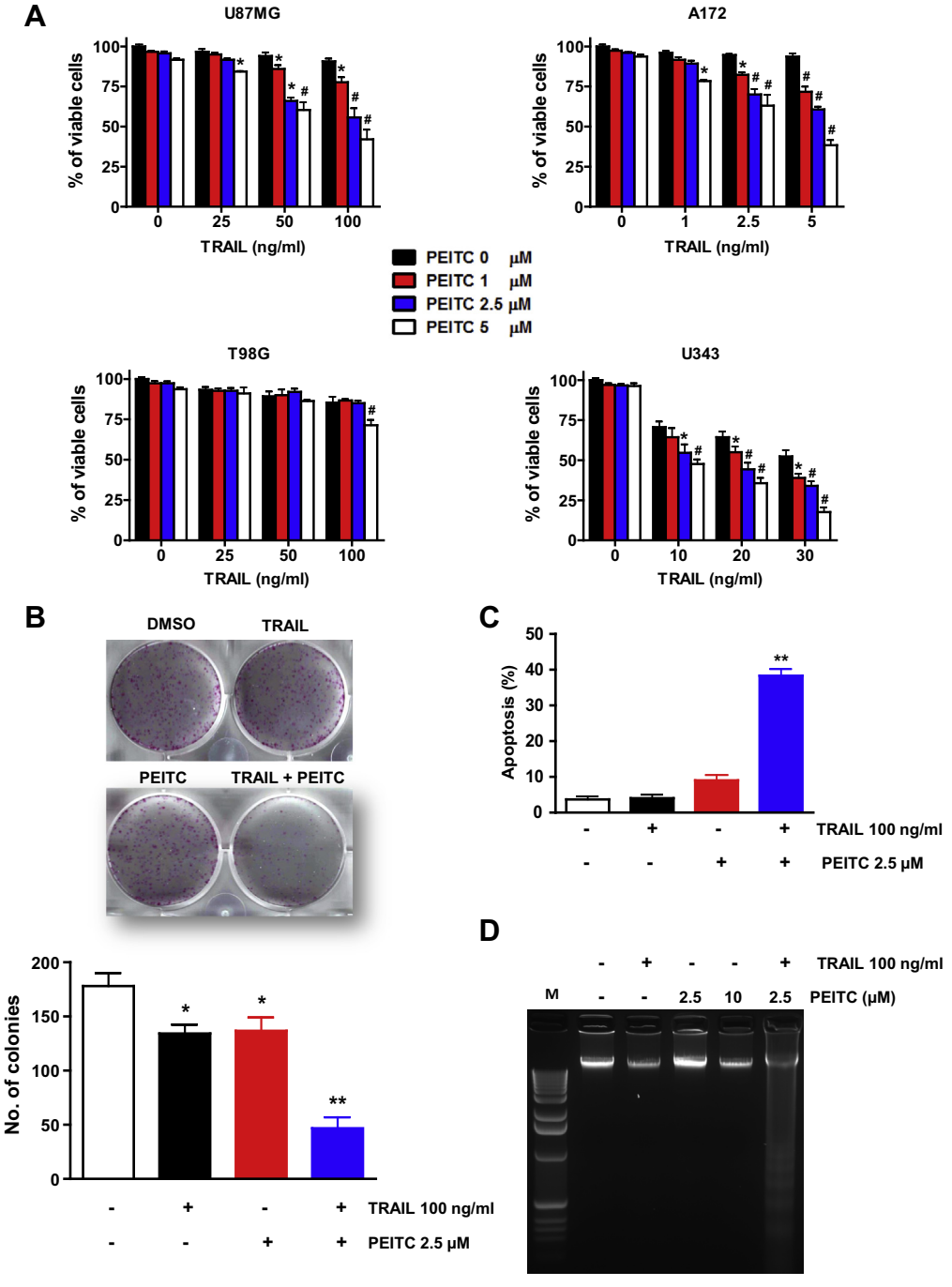

Fig. 1. Subtoxic doses of PEITC sensitize various human glioma cells to TRAIL-induced apoptosis. (A) Four different human glioma cell lines were untreated or treated with PEITC for 30 min and further treated with TRAIL for 24 h at the indicated concentration. Cellular viability was assessed using Trypan blue assay. Columns indicate average of three individual experiments; bars represents ±SD; $^{*}p < 0.05$, compared with untreated cells; $^{\#}p < 0.01$, compared with TRAIL-treated cells. (B) cells were treated with 2.5 μM PEITC for 12 h, washed with PBS to remove PEITC, and then treated with TRAIL (100 ng/mL) for 12 h. The cells were then reseeded in 6-well plates and allowed to form colonies for 14 d, after which they were stained with crystal violet, as described in Materials and Methods. (C) U87MG cells treated with 2.5 μM PEITC alone, 100 ng/ml TRAIL alone, or PEITC + TRAIL for 24 h. Apoptosis was analyzed by the annexin-V assay as described in materials and methods. Error bars represent the mean + SE from three separate experiments. Asterisk ** represents a statistically significant difference between control and TRAIL-treated cells at $p < 0.001$. (D) Fragmented DNA was extracted from the treated cells and analyzed on 1.5% agarose gel.

Biotechnology (Santa Cruz, CA, USA). Anti-catalase and anti-caspase-8 antibody was purchased from Cell Signaling (Beverly, MA, USA). Anti-caspase-9 antibody was purchased from Upstate Biotechnology (Lake Placid, NY, USA). Anti-cytochrome C, anti-DR4, and anti-DR5 were purchased from R&D Systems (Plymouth Meeting, PA, USA). Anti-PARP antibody was purchased from Biomol Research Laboratory (Plymouth Meeting, PA, USA). Anti-actin antibody was purchased from MP Biomedicals (Solon, OH, USA). For the secondary antibodies, anti-mouse-IgG-HRP and anti-rabbit-IgG-HRP were purchased from Cell Signaling. Adenovirus expressing luciferase and catalase were purchased from Vector Biolabs (Philadelphia, PA, USA).

2.2. Cell lines and cell culture

U87MG, A172, U343, and T98G cells were obtained from the American Type Culture Collection (ATCC) (Rockville, MD, USA). The culture medium used throughout these experiments was Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 100 mg/ml gentamycin. The dishes containing cells were kept in a 37 °C humidified incubator with 5% CO_2.

2.3. Determination of cell viability

One day prior to the experiment, cells were plated into 60-mm dishes at a density of 5×10^4 cells/plate in 5 ml tissue culture medium in triplicate. For trypan blue exclusion assay, trypsinized cells were pelleted and resuspended in 0.5 ml of 0.4% trypan blue solution and 0.5 ml of phosphate-buffered saline solution (PBS). The samples were mixed thoroughly, incubated at room temperature for 10 min, and examined under a light microscope. At least 500 cells were counted for each survival determination.

2.4. DNA fragmentation assay

After treatment with PEITC, TRAIL, or a combination of PEITC and TRAIL for 24 h, U87MG cells were lysed in buffer containing 10 mmol/l Tris (pH 7.4), 150 mmol/l NaCl, 5 mmol/l EDTA, and 0.5% Triton X-100 for 30 min on ice. Lysates were vortexed and cleared by centrifugation at 10,000*g* for 20 min. Fragmented DNA in the supernatant was extracted with an equal volume of a neutral phenol:chloroform:isoamyl alcohol mixture (25:24:1) and analyzed electrophoretically on 2% agarose gels containing 0.1 μg/ml ethidium bromide.

2.5. Clonogenic assay

Treated and untreated cells were plated in 6-well-plates at the density of 1000/well and cultured for 14 days. The colonies were stained with 1.0% crystal violet for 30 s after fixation with 10% formaldehyde for 5 min. The number of colonies was counted by visual inspection.

2.6. Measurement of ROS generation

ROS generation in control and PEITC-treated cells was measured by flow cytometry following staining with chloro methyl derivative of CMH_2DCFDA (2.5 μM) or MitoSOX (2.5 μM). ROS was detected by flow cytometry as reported previously [9]. CM-H_2-

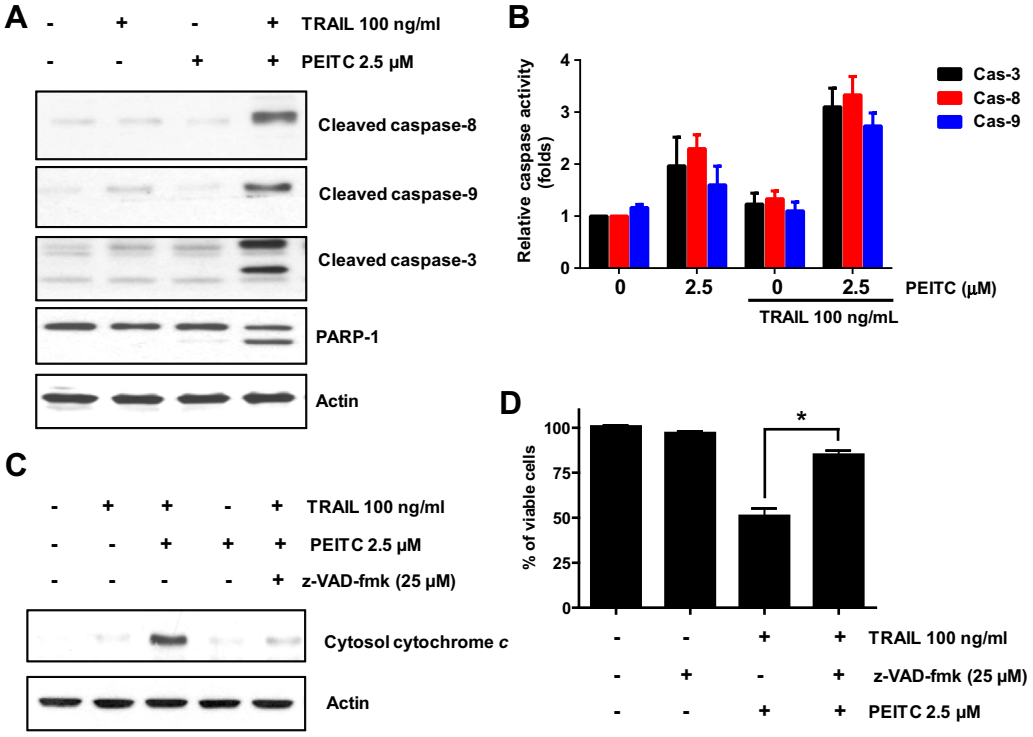

Fig. 2. PEITC enhances TRAIL-induced caspase activation and apoptosis. (A) U87MG cells treated with 2.5 μM PEITC alone, 100 ng/ml TRAIL alone, or PEITC + TRAIL for 24 h. Lysates containing equal amounts of protein (20 μg) were separated by SDS–PAGE and immunoblotted with anti-PARP-1, anti-caspase-8, anti-caspase-9, or anti-caspase-3 antibody. (B) U87MG cells treated with 2.5 μM PEITC alone, 100 ng/ml TRAIL alone, or PEITC + TRAIL for 24 h. Relative caspase activities were determined by the manufacturer's protocol. (C) Cells were treated with PEITC alone, TRAIL alone, or PEITC in combination with TRAIL for 24 h. Lysates containing equal amounts of protein (20 μg) were separated by SDS–PAGE and immunoblotted with anti-cytochrome *c* antibody. Actin was used to confirm the equal amount of proteins loaded in each lane. (D) To examine the effect of the inhibition of pan-caspase, U87MG cells were pretreated with 25 μM z-VAD-fmk for 30 min and further treated with PEITC + TRAIL for 24 h. Cellular viability was assessed using an MTT assay. Data are expressed as overall mean ± SE from three independent experiments. Statistical significance was determined by two-way ANOVA test (*$p < 0.05$ vs. control).

DCFDA is mainly oxidized by H_2O_2 and hydroxyl radical; MitoSOX is oxidized by superoxide anion. Briefly, the desired cell line was seeded in six-well plates (1×10^5 cells per well), allowed to attach overnight and exposed to DMSO (control) or desired concentrations of PEITC for specified time periods. The cells were stained with 2.5 μM CMH_2DCFDA and MitoSOX for 30 min at 37 °C, and fluorescence was detected by fluorescence microscope. Alternatively, the fluorescence intensity of dichlorofluorescein in cells was determined using the flow cytometer (Becton Dickinson and Co. Ltd.).

2.7. Analysis of cell surface DR4 and DR5

Treated and untreated cells were detached with Trypsin–EDTA and were washed three times with PBS wash buffer supplemented with 0.25% BSA. Cells were resuspended in 500 μl PBS, stained with primary antibody (1 μg/ml), and incubated for 1 h at 4 °C. Unreacted antibody was removed by washing the cells twice with PBS buffer. Cells were stained with secondary antibody conjugated with fluorescein isothiocyanate (FITC) and incubated for 30 min at 4 °C. Unbound FITC-conjugated antibody was washed twice with PBS. Cells were resuspended in 500 μl PBS. Surface expression of DR4 and DR5 was determined by flow cytometry.

2.8. Immunocytochemistry

U87MG cells were either treated or not with 2.5 μM PEITC for 16 h, stained with antibodies against DR5 and processed for fluorescence microscopy. In brief, cells were fixed with 4% paraformaldehyde, incubated with monoclonal antibodies recognizing DR5 (1:200 in 1% BSA buffer (PBS containing 0.1% Tween 20)) followed by Alexa Fluor 488-conjugated anti-rabbit IgG (1:200 in 1% BSA buffer). Visualization was performed with Alexa fluorophore-conjugated secondary antibodies.

2.9. Analysis of apoptosis by flow cytometry

Treated and untreated cells were prepared and analyzed by flow cytometry as described previously [11]. Briefly, cells were stained using Annexin V-FITC Detection Kit II (BD Biosciences Pharmingen, San Diego, CA) according to the manufacturer's recommendations. Stained cells were analyzed by fluorescent-activated cell sorting on a FACScan flow cytometer (Becton Dickinson, Franklin Lakes, NJ).

2.10. RNA interference by siRNA of DR5

To knockdown DR5 or survivin gene expression, DR5 siRNA, or control siRNA were purchased from Santa Cruz Biotechnology. Human glioma cells were seeded in six-well plate at a density of 2×10^5 cells per well in 2 ml complete RPMI 1640 growth medium. Twenty-four hours later, when cells reached 80% confluence, growth medium was replaced with 0.8 ml/well Opti-MEM transfection medium (Invitrogen) and 0.2 ml siRNA transfection mix. Transfections were carried out according to Invitrogen's oligofectamine protocol. After transfection, cells were incubated for 48 h at 37 °C in a 5% CO_2 humidified atmosphere and the inference of DR5 expression was confirmed by immunoblotting using anti-DR5 antibody.

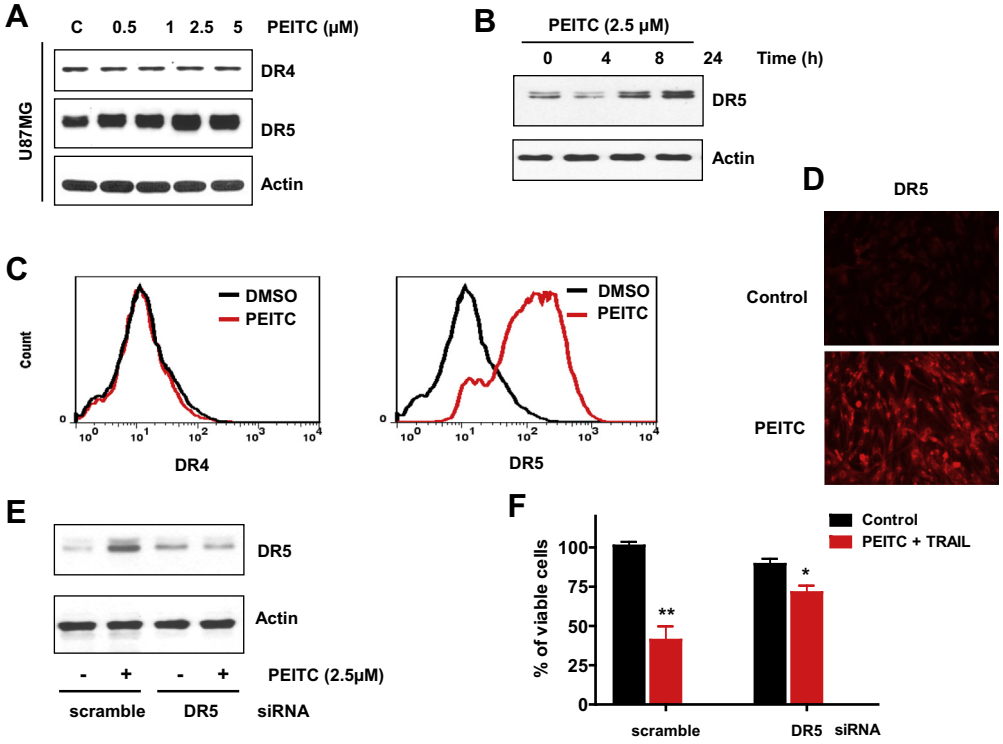

Fig. 3. PEITC can regulate the expression of death receptor DR5, but not DR4. (A and B) U87 cells were treated with indicated PEITC doses (0, 0.5, 1, 2.5, 5 μM) for 24 h or 2.5 μM PEITC at an indicated time (0, 4, 8, 24 h). Western blotting analysis was performed with DR4 and DR5 antibodies. (C and D) U87 cells were incubated with DMSO or PEITC (2.5 μM) for 24 h and stained with DR4 and DR5 antibodies, followed by FACS analysis (left panels) and immunocytochemistry (right panels). (E) U87 cells were transfected with siRNA-DR5 or siRNA-scramble (control). After treatment with PEITC for 24 h, Western blot analysis demonstrated that DR5 expression decreased apoptosis compared with control. (F) Cell viability was determined using the trypan blue dye exclusion assay. Error bars represent the mean ± SE from three separate experiments. Significant difference between siRNA-scramble (control) and siRNA-DR5 transfected cells at $^{*}P < 0.05$; $^{**}P < 0.01$. These results are representative of data obtained from at least three independent experiments.

2.11. Western blot analysis

Whole cell protein lysates were prepared and analyzed by Western blotting as described previously [11].

2.12. Statistical analysis

Statistical analysis was carried out using Graphpad InStat 6 software (GraphPad Software, Inc., San Diego, CA, USA). Results were considered statistically significant at $P < 0.05$.

3. Results

3.1. Subtoxic doses of PEITC effectively sensitize human glioma cells to TRAIL-induced apoptosis

To assess the ability of PEITC to act as a sensitizer of malignant glioma cells to the apoptotic effects of TRAIL, we first examined the effect of TRAIL with and without PEITC on the viability of a number of different glioma cell lines. We found that U87MG, A172, and T98G cells were relatively resistant to TRAIL over a range of doses, whereas U343 cells were somewhat sensitive to TRAIL. PEITC alone induced a modest level of glioma cell death (<20%) at concentrations up to 5 μM. In contrast, the viability of glioma cells was significantly reduced by combined treatment with a fixed concentration of TRAIL and varied concentrations of PEITC or conversely with a fixed PEITC concentration and varied concentrations of TRAIL (Fig. 1A). The typical morphological changes of apoptotic cell death were observed under phase-contrast microscopy (data not shown). Next, we determined whether PEITC can enhance the effect of TRAIL in a long-term colony formation assay. We found that PEITC or TRAIL alone was minimally effective in inhibiting colony formation of U87 cells but that the combination treatment significantly suppressed the colony-forming ability of these tumor cells (Fig. 1B, upper and lower). Similar results were observed by Annexin V and PI staining assay. Data from FACS analysis show that apoptotic death occurred during combined treatment with TRAIL and PEITC (Fig. 1C). Cotreatment of U87cells with PEITC and TRAIL resulted in a markedly increased accumulation of sub-G1-phase cells and a typical ladder pattern of internucleosomal fragmenta-

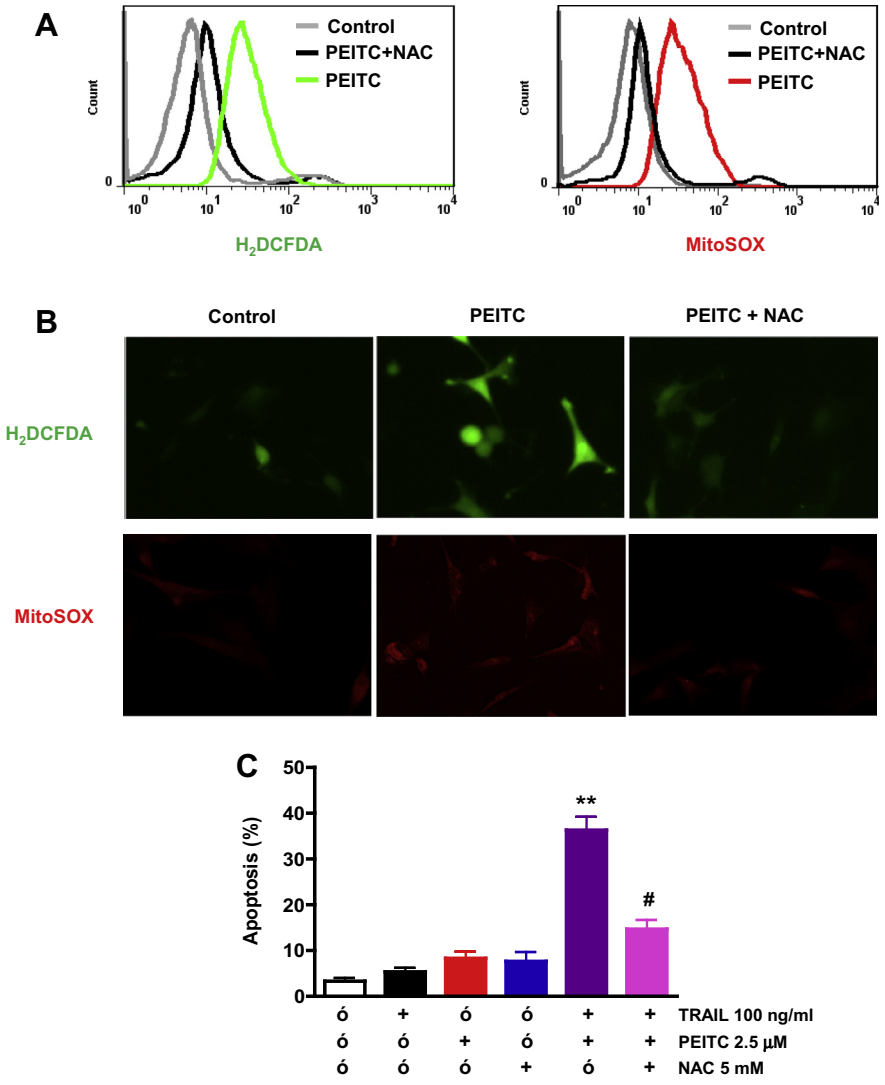

Fig. 4. PEITC induces elevation of the intercellular ROS level. (A and B) To determine the intracellular content of peroxides and anion superoxide, U87MG cells were loaded with H2DCFDA and MitoSOX, respectively, and fluorescence was measured by flow cytometry. U87MG cells were loaded with fluorescence-dye and further stimulated with PEITC (2.5 μM) in the presence or absence of NAC (5 mM). After 4 h, H2DCFDA and MitoSOX fluorescence was visualized using a confocal microscope. (C) Cells were pretreated with 4 mM NAC for 2 h followed by treatment with 2.5 μM PEITC and/or 100 ng/ml TRAIL for 24 h. Survival was analyzed by the annexin-V assay as described in materials and methods. Error bars represent the mean + SE from three separate experiments. Asterisk represents a statistically significant difference between control and TRAIL-treated cells at $^{*}p < 0.001$. $^{\#}p < 0.01$, compared with TRAIL-treated cells.

tion, compared with U87 cells treated with PEITC or TRAIL alone (Fig. 1D). Taken together, our results indicate that PEITC can enhance TRAIL-induced apoptosis.

3.2. PEITC potentiates TRAIL-mediated apoptosis via the extrinsic signal pathways

Since activation of caspases is a key hallmark of apoptosis [12], we investigated the effect of PEITC on the activation of caspases and cleavage of PARP in the presence of TRAIL. Western blot analyses showed that PEITC substantially increases TRAIL-induced activation of caspases-3, -8 and -9, thus leading to enhanced PARP cleavage in U87MG cells (Fig. 2A). We also found that combined treatment with PEITC and TRAIL resulted in the activation of capase-3, -8 and -9 in U87MG cells (Fig. 2A). In addition, pretreatment with pan-caspase inhibitor z-VAD-fmk significantly attenuated combined treatment-induced cell death, little cytotoxicity (<10%) was observed in cells treated with TRAIL or PEITC alone (Fig. 2C). In a parallel experiment, we verified by PI and annexin-V staining whether combined treatment-mediated apoptosis is closely related to receptor-dependent caspase-8 activation. We found that treatment with PEITC or TRAIL alone hardly induced apoptosis compared to control (Fig. 2B). However, the combined treatment enhanced 25.8% of sub-G_1 populations and 20.7% of annexin-V^+ cells, respectively (data now shown). Apoptosis induced by the combined treatment was also significantly blocked by pretreatment with caspase-8 inhibitor z-IETC-fmk (Fig. 2D). In addition, this process usually was also blocked by accompanied by the release of cytochrome *c* from the mitochondria into the cytosol, pretreatment with z-IETD-fmk (Fig. 2C). These results indicate that the combined treatment with PEITC and TRAIL enhances TRAIL-induced cell death in a cell-type non-specific manner via caspase activation.

3.3. PEITC induces expression of DR5 to sensitize TRAIL-induced apoptosis

In order to investigate how PEITC enhances TRAIL-induced apoptosis, we examined the expression of DR5 and DR4 on U87MG cells. Treatment of U87MG cells with PEITC for 24 h resulted in an increased expression of DR5 in a dose and time-dependent manner, but did not affect the levels of DR4 (Fig. 3A and B). The analysis of FACS and immunofluorescence staining also showed that PEITC induced surface expression of DR5, but not DR4 in U87MG cells (Fig. 3C and D). Thus, to determine the role of DR5 in the combined treatment-induced apoptosis, we pretreated with blocking antibodies or specific siRNA to DR5 to down-regulate the expression of DR5 (Fig. 3E). We also found that the combined treatment-induced cell death was effectively abolished in cells transfected with DR5 siRNA (Fig. 3F), whereas treatment with control siRNA had no effect. These results indicate that PEITC-induced DR5 up-regulation plays a critical role in the enhancement of TRAIL sensitivity.

3.4. Upregulation of DR5 by PEITC appears to be dependent on the formation of reactive oxygen metabolites

As shown in Fig. 4A, treatment with PEITC markedly increased the H_2DCFDA- and MitoSOX-derived fluorescence. This PEITC-mediated increase in fluorescence was markedly inhibited by pretreatment with NAC, a thiol antioxidant, which is known to function as both a redox buffer and a reactive oxygen intermediate scavenger. Production of ROS by PEITC was further confirmed using fluorescence microscopy. U87MG cells treated with PEITC displayed intense fluorescence inside the cell on staining with H_2DCFDA- or MitoSOX-dye (Fig. 4B), demonstrating the intracellular accumulation of ROS. This increased fluorescence derived from H_2DCFDA or MitoSOX in PEITC-treated U87MG cells was also markedly reduced by NAC (Fig. 4B). We also used NAC to examine the role of ROS in PEITC plus TRAIL-induced apoptosis. Pretreatment of U87MG cells with NAC markedly blocked PEITC plus TRAIL-induced apoptosis (Fig. 4C). These data clearly indicate that the blocking of PEITC plus TRAIL-induced apoptosis by NAC is associated with ROS generation.

4. Discussion

In the present study, we show for the first time that subtoxic doses of PEITC effectively sensitize different glioma cell lines to TRAIL-induced apoptosis. Accumulation of intercellular ROS leads to disruption of mitochondrial membrane potential, release of cytochrome *c* into the cytosol with subsequent activation of the caspase cascade, and finally to programmed cell death through apoptosis. Recently it has been suggested that oxidative stress has a pivotal role as a common mediator of apoptosis [9,13,14]. We and other groups reported that PEITC generates ROS intermediates in cancer cells [15,16]. In this study, we demonstrate that PEITC-induced ROS can induce DR5 upregulation through transcriptional control, although it remains to be clarified how ROS increase the transcriptional activity of DR5 promoter (data not shown). Several studies have reported that PEITC can induce apoptosis in a p53-dependent or independent manner [16–18]. In addition, PEITC has been reported to inhibit cytokine gene expression by PI3K/AKT inhibition [19]. In our study we investigated the effect of PEITC on TRAIL-induced cell death. Combined treatment with PEITC and TRAIL enhanced DNA fragmentation, caspase-3 activation and PARP cleavage. The TRAIL-induced sensitized apoptosis by PEITC was effectively inhibited by a general inhibitor of caspases, z-VAD-fmk, suggesting that this cell death is dependent on caspases.

TRAIL induces apoptosis by interacting with two death-inducing receptors, DR4 and DR5. The death receptors DR4 and/or DR5 have been demonstrated to play a crucial role in synergistic cytotoxicity associated with TRAIL and chemotherapeutic agents [9,20–22]. In the present study, we show for the first time that PEITC is an effective TRAIL sensitizer that acts via a DR5-upregulation mechanism in various glioma cell lines. Small silencing RNA-mediated DR5 suppression significantly attenuated the cell death induced by combined treatment with PEITC and TRAIL, demonstrating the functional significance of DR5 in this cell death.

We found that ROS generation occurs during treatment with PEITC. ROS, including the superoxide anion, hydrogen peroxide and hydroxyl radical, are known to mediate apoptosis induced by some cancer chemopreventive and therapeutic agents [23–26]. Intracellular ROS may interact with cellular membrane lipids, proteins, and DNA and cause oxidative injury [27,28]. Recently, Xiao et al. [16] also reported that treatment with PEITC induces apoptosis in prostate cancer cells and that the initial signal for PEITC-induced apoptosis is derived from ROS. These results suggest that ROS act as upstream signaling molecules for the initiation of PEITC-induced p53 expression and are critical for the sensitization of the cells to TRAIL-induced apoptosis.

Conflict of interest

The authors state no conflict of interest.

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References

[1] G.N. Fuller, The WHO classification of tumours of the central nervous system, 4th edition, Arch. Pathol. Lab. Med. 132 (2008) 906.
[2] E.S. Henson, J.B. Johnston, S.B. Gibson, The role of TRAIL death receptors in the treatment of hematological malignancies, Leukemia Lymphoma 49 (2008) 27–35.
[3] T. Ohtsuka, D. Buchsbaum, P. Oliver, S. Makhija, R. Kimberly, T. Zhou, Synergistic induction of tumor cell apoptosis by death receptor antibody and chemotherapy agent through JNK/p38 and mitochondrial death pathway, Oncogene 22 (2003) 2034–2044.
[4] A.F. Kabore, J. Sun, X. Hu, K. McCrea, J.B. Johnston, S.B. Gibson, The TRAIL apoptotic pathway mediates proteasome inhibitor induced apoptosis in primary chronic lymphocytic leukemia cells, Apoptosis 11 (2006) 1175–1193.
[5] M.M. Keane, S.A. Ettenberg, M.M. Nau, E.K. Russell, S. Lipkowitz, Chemotherapy augments TRAIL-induced apoptosis in breast cell lines, Cancer Res. 59 (1999) 734–741.
[6] H. Yagita, K. Takeda, Y. Hayakawa, M.J. Smyth, K. Okumura, TRAIL and its receptors as targets for cancer therapy, Cancer Sci. 95 (2004) 777–783.
[7] J.M. Kuijlen, E. Bremer, J.J. Mooij, W.F. den Dunnen, W. Helfrich, Review: on TRAIL for malignant glioma therapy?, Neuropathol Appl. Neurobiol. 36 (2010) 168–182.
[8] A. Panner, C.A. Crane, C. Weng, A. Feletti, A.T. Parsa, R.O. Pieper, A novel PTEN-dependent link to ubiquitination controls FLIPS stability and TRAIL sensitivity in glioblastoma multiforme, Cancer Res. 69 (2009) 7911–7916.
[9] D.H. Lee, J.G. Rhee, Y.J. Lee, Reactive oxygen species up-regulate p53 and Puma; a possible mechanism for apoptosis during combined treatment with TRAIL and wogonin, Br. J. Pharmacol. 157 (2009) 1189–1202.
[10] D.O. Moon, C.H. Kang, S.H. Kang, Y.H. Choi, J.W. Hyun, W.Y. Chang, H.K. Kang, Y.S. Koh, Y.H. Maeng, Y.R. Kim, G.Y. Kim, Capsaicin sensitizes TRAIL-induced apoptosis through Sp1-mediated DR5 up-regulation: involvement of Ca(2+) influx, Toxicol. Appl. Pharmacol. 259 (2012) 87–95.
[11] D.H. Lee, T.H. Lee, C.H. Jung, Y.H. Kim, Wogonin induces apoptosis by activating the AMPK and p53 signaling pathways in human glioblastoma cells, Cell Signal. 24 (2012) 2216–2225.
[12] H. Pan, C. Yin, T. Van Dyke, Apoptosis and cancer mechanisms, Cancer Surv. 29 (1997) 305–327.
[13] Y.S. Hori, A. Kuno, R. Hosoda, Y. Horio, Regulation of FOXOs and p53 by SIRT1 modulators under oxidative stress, PLoS ONE 8 (2013) e73875.
[14] Y.J. Zhong, S.P. Liu, R.A. Firestone, Y.P. Hong, Y. Li, Anticancer effects of AcPheLysPABCdoxorubicin via mitochondriacentered apoptosis involving reactive oxidative stress and the ERK1/2 signaling pathway in MGC803 cells, Oncol. Rep. 30 (2013) 1681–1686.
[15] B. Gupta, L. Chiang, K. Chae, D.H. Lee, Phenethyl isothiocyanate inhibits hypoxia-induced accumulation of HIF-1alpha and VEGF expression in human glioma cells, Food Chem. 141 (2013) 1841–1846.
[16] D. Xiao, A.A. Powolny, M.B. Moura, E.E. Kelley, A. Bommareddy, S.H. Kim, E.R. Hahm, D. Normolle, B. Van Houten, S.V. Singh, Phenethyl isothiocyanate inhibits oxidative phosphorylation to trigger reactive oxygen species-mediated death of human prostate cancer cells, J. Biol. Chem. 285 (2010) 26558–26569.
[17] N.Y. Tang, Y.T. Huang, C.S. Yu, Y.C. Ko, S.H. Wu, B.C. Ji, J.S. Yang, J.L. Yang, T.C. Hsia, Y.Y. Chen, J.G. Chung, Phenethyl isothiocyanate (PEITC) promotes G2/M phase arrest via p53 expression and induces apoptosis through caspase- and mitochondria-dependent signaling pathways in human prostate cancer DU 145 cells, Anticancer Res. 31 (2011) 1691–1702.
[18] S.H. Kim, S.V. Singh, P53-Independent apoptosis by benzyl isothiocyanate in human breast cancer cells is mediated by suppression of XIAP expression, Cancer Prev. Res. (Phila) 3 (2010) 718–726.
[19] T. Okubo, K. Washida, A. Murakami, Phenethyl isothiocyanate suppresses nitric oxide production via inhibition of phosphoinositide 3-kinase/Akt-induced IFN-gamma secretion in LPS-activated peritoneal macrophages, Mol. Nutr. Food Res. 54 (2010) 1351–1360.
[20] H. Cao, Y. Cheng, L. You, J. Qian, W. Qian, Homoharringtonine and SAHA synergistically enhance apoptosis in human acute myeloid leukemia cells through upregulation of TRAIL and death receptors, Mol. Med. Rep. 7 (2013) 1838–1844.
[21] B. Sung, S. Prasad, J. Ravindran, V.R. Yadav, B.B. Aggarwal, Capsazepine, a TRPV1 antagonist, sensitizes colorectal cancer cells to apoptosis by TRAIL through ROS-JNK-CHOP-mediated upregulation of death receptors, Free Radical Biol. Med. 53 (2012) 1977–1987.
[22] S. Prakobwong, S.C. Gupta, J.H. Kim, B. Sung, P. Pinlaor, Y. Hiraku, S. Wongkham, B. Sripa, S. Pinlaor, B.B. Aggarwal, Curcumin suppresses proliferation and induces apoptosis in human biliary cancer cells through modulation of multiple cell signaling pathways, Carcinogenesis 32 (2011) 1372–1380.
[23] H. Luo, A. Yang, B.A. Schulte, M.J. Wargovich, G.Y. Wang, Resveratrol induces premature senescence in lung cancer cells via ROS-mediated DNA damage, PLoS ONE 8 (2013) e60065.
[24] K. Chandra-Kuntal, J. Lee, S.V. Singh, Critical role for reactive oxygen species in apoptosis induction and cell migration inhibition by diallyl trisulfide, a cancer chemopreventive component of garlic, Breast Cancer Res. Treat. 138 (2013) 69–79.
[25] H.Y. Khan, H. Zubair, M.F. Ullah, A. Ahmad, S.M. Hadi, A prooxidant mechanism for the anticancer and chemopreventive properties of plant polyphenols, Curr. Drug Targets 13 (2012) 1738–1749.
[26] M.J. Kim, D.H. Kim, H.K. Na, Y.J. Surh, TNF-alpha induces expression of urokinase-type plasminogen activator and beta-catenin activation through generation of ROS in human breast epithelial cells, Biochem. Pharmacol. 80 (2010) 2092–2100.
[27] W.J. Mai, J.L. Yan, L. Wang, Y. Zheng, Y. Xin, W.N. Wang, Acute acidic exposure induces p53-mediated oxidative stress and DNA damage in tilapia (*Oreochromis niloticus*) blood cells, Aquat. Toxicol. 100 (2010) 271–281.
[28] Y. Saitoh, A. Miyanishi, H. Mizuno, S. Kato, H. Aoshima, K. Kokubo, N. Miwa, Super-highly hydroxylated fullerene derivative protects human keratinocytes from UV-induced cell injuries together with the decreases in intracellular ROS generation and DNA damages, J. Photochem. Photobiol. B 102 (2011) 69–76.