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Involvement of ERK1/2-mediated ELK1/CHOP/DR5 pathway in 6-(methylsulfinyl)hexyl isothiocyanate-induced apoptosis of colorectal cancer cells

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ABSTRACT

6-(Methylsulfinyl)hexyl isothiocyanate (6-MSITC) is a major bioactive compound in Wasabi. Although 6-MSITC is reported to have cancer chemopreventive activities in rat model, the molecular mechanism is unclear. In this study, we investigated the anticancer mechanisms using two types of human colorectal cancer cells (HCT116 $p53^{+/+}$ and $p53^{-/-}$). 6-MSITC caused cell cycle arrest in G_2/M phase and induced apoptosis in both types of cells in the same fashion. Signaling data revealed that the activation of ERK1/2, rather than *p53*, is recruited for 6-MSITC-induced apoptosis. 6-MSITC stimulated ERK1/2 phosphorylation, and then activated ERK1/2 signaling including ELK1 phosphorylation, and upregulation of C/EBP homologous protein (CHOP) and death receptor 5 (DR5). The MEK1/2 inhibitor U0126 blocked all of these molecular events induced by 6-MSITC, and enhanced the cell viability in both types of cells in the same manner. These results indicated that ERK1/2-mediated ELK1/CHOP/DR5 pathway is involved in 6-MSITC-induced apoptosis in colorectal cancer cells.

Abbreviations: CHOP: C/EBP homologous protein; DR5: death receptor 5; ELK1: ETS transcription factor; ERK1/2: extracellular signal-regulated kinase 1/2; JNK: Jun-*N*-terminal kinase; MAPK: mitogen-activated protein kinase; MEK1/2: MAP/ERK kinase 1/2; 6-MSITC: 6-(methylsulfinyl)hexyl isothiocyanate; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PARP: poly(ADP-ribose) polymerase.

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Wasabi [*Wasabia japonica* (Miq.) Matsum.] is a very popular pungent spice in Japan, usually used as flavor for Sashimi. Several lines of studies have reported that Wasabi has anti-microbial activity [1] and anti-carcinogenic effect in *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG)-induced rat gastric carcinogenesis [2]. The major bioactive component in Wasabi was identified as 6-(methylsulfinyl)hexyl isothiocyanate (6-MSITC), an analogues of sulforaphane [3–5]. Oral administration of 6-MSITC in colon carcinogenesis model of rat revealed that 6-MSITC could suppress the number of colonic aberrant crypt foci induced by 1, 2-dimethylhydrazine [6]. These results suggest that 6-MSITC has a chemopreventive potency for colon carcinogenesis, however, the underlying mechanisms are not clear.

Apoptosis is highly regulated programmed cell death [7]. It is characterized by cellular morphological change, DNA fragmentation, and activation of caspase cascades [8]. Apoptosis has been known to be occurred by various stimuli, lead to cell death without severe inflammation [9,10]. Especially, many cancer chemopreventive compounds and cancer chemotherapeutic agents have been developed to induce apoptosis of serious damaged cells or tumor cells for cancer chemoprevention [11] and cancer therapy [12].

Although there are many cell signaling pathways to regulate cell viability and death, P53, a tumor suppressor protein, has been reported to play a central role in cell cycle arrest and apoptosis [13]. It is well known that many chemotherapeutic drugs revealed their anticancer effects by activating P53 with genetoxic stress [14]. On the other hand, the RAF-MEK-ERK pathway, in general, represents a major signaling pathway to promote cell viability via the inhibition of apoptosis [15,16]. However, a proapoptotic role of ERK signaling has also been reported in some studies [17]. A case documented well is that ERK1/2 signaling positively regulates the expression of death receptor 5 (DR5) to induce apoptosis [18–20]. ERK1/2 activation causes the phosphorylation of ELK1 and upregulation of C/EBP homologous protein (CHOP) through ERK/RSK-mediated ATF4 activation [19]. The phosphorylated ELK1 and increased CHOP bind the promoter (−323/−308, −276/−264) of *dr5* gene to co-operate its transactivation [18,19].

Based on the chemopreventive effect of Wasabi 6-MSITC on colorectal carcinogenesis in rat model,

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and the roles of *p53* on apoptosis induction in cancer cells, we used two types of human colorectal cancer cells (HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$) to investigate the anticancer activity and molecular mechanisms of Wasabi 6-MSITC in the present study.

Materials and methods

Materials

6-MSITC was kindly provided by Kinjirushi Co., Ltd. (Nagoya, Japan). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and the antibody against β-actin were purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA). SP600125, SB202190, U0126 and the antibodies against phospho-P53 (Ser15), caspase-3, caspase-8, PARP, phospho-JNK, phospho-p38, phospho-ERK1/2, JNK, p38, ERK1/2, phospho-ELK1, CHOP and DR5 were from Cell Signaling Technology, Inc. (Danvers, MA, USA). The antibody against P53 was from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA).

Cell culture

Human colorectal cancer cell lines HCT116 $p53^{+/+}$ were obtained from the American Type Culture Collection (Manassas, VA, USA) and HCT116 $p53^{-/-}$ cells were kindly supplied by Dr. Bert Vogelstein (Johns Hopkins Medical Institute, Baltimore, MD, USA). The cells were cultured at 37°C in a 5% CO_2 atmosphere in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin glutamine (PSG) for 24 h, and then treated by 6-MSITC in indicated times and doses.

Western blotting analysis

The cells were lysed with modified RIPA buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.25% Na-deoxycholate, 1 mM sodium fluoride, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, and proteinase inhibitor cocktail (Nacalai Tesque, Inc., Kyoto, Japan). The lysates were homogenized in an ultrasonicator for 10 s three times and incubated on ice for 30 min. The homogenates were centrifuged at 14,000 × g for 30 min and the supernatants were collected. The protein concentration was determined by Bio-Rad Protein Assay Dye Reagent Concentrate (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Equal amounts of lysate protein were run on SDS-PAGE and electrophoretically transferred to PVDF membrane (GE Healthcare UK Ltd., Amersham, England). The membrane was first blocked with TBST buffer (500 mM NaCl, 20 mM Tris-HCl (pH 7.4), and 0.1% Tween 20) containing 5% nonfat dry milk, and then incubated with specific antibodies overnight at 4°C and HRP-conjugated secondary antibodies for another 1 h. Bound antibodies were detected using the ECL system and relative amounts of proteins associated with specific antibody were quantified using Lumi Vision Imager software (TAITEC Co., Ltd., Saitama, Japan)

Cell cycle analysis

The cell cycle phases were determined by a flow cytometric analysis. After treatment with 20 μM 6-MSITC for 24 h, HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cells were fixed in 70% ethanol at −20°C overnight and then resuspended in PBS. The cells were stained in 1 mL PBS containing 20 μg/mL RNase and 50 μg/mL propidium iodide (PI) for 30 min at room temperature. Fluorescence emitted from the propidium-DNA complex was analyzed with the flow cytometry (CyFlow®, Sysmex Partec GmbH, Görlitz, Germany).

Cell viability assay

The cell viability rate was measured by an MTT assay. HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ (7.0×10^3/well) cells were plated into each well of 96-well plates. The cells were treated with the indicated concentrations of 6-MSITC for 48 h. MTT solution was then added to each well and incubated for another 4 h. The resulting MTT-formazan product was dissolved by the addition of 0.04 N HCl-isopropanol solutions. The amount of formazan was determined by measuring the absorbance at 595 nm with Multiskan™ FC (Thermo Scientific™, Waltham, MA, USA). The cell viability was expressed as the optical density ratio of the treatment to control.

Determination of apoptotic cells by flow cytometry

Quantitation of apoptotic cell death was assessed by FITC Annexin V Apoptosis Detection Kit I (BD Biosciences, San Diego, CA, USA) according to the manufacturer's manual. In brief, after treatment with each sample for 48 h, HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cells were suspended in 100 μL of binding buffer and then incubated with FITC Annexin V and PI staining solution for 15 min. The cells were analyzed at FL1 (530 nm) and FL3 (630 nm) with the flow cytometry (CyFlow®, Sysmex Partec GmbH, Görlitz, Germany).

Statistical analysis

The data represent the mean ± SD. All data were statistically analyzed by two-way or three-way analysis of variance (ANOVA), and then Tukey–Kramer test was used as a post hoc comparison in Figures 2(b) and 3(b). The results of two-way and three-way ANOVA were indicated as a table in the upper side of

graphs. A probability of $p < 0.01$ was considered as significant.

Results

6-MSITC induces cell cycle arrest in G_2/M phase in a p53-independent manner

The tumor suppressor protein, P53, has been reported to play a central role in cell cycle arrest and apoptosis [13]. The phosphorylation of P53 on Ser15 occurs in cell cycle arrest [21] and apoptosis induction [22]. To examine whether 6-MSITC induces the activation of P53, HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cells were treated with 20 μM 6-MSITC for 0–48 h. As shown in Figure 1(a), an increased phosphorylation of P53 protein on Ser15 was observed from 24–48 h in HCT116 $p53^{+/+}$ cells, but not in HCT116 $p53^{-/-}$ cells due to loss of *p53* gene. To elucidate whether 6-MSITC-induced phosphorylation of P53 in HCT116 $p53^{+/+}$ cells influences the viability, we examined the cell distribution in different cell cycles with flow cytometric analysis. As shown in Figure 1(b,c), a significant increase of G_2/M phase cells were observed in both types of cells after treatment with 6-MSITC for 24 h. Two-way ANOVA results indicated that *p53* had no significant effect on cell cycle distribution induced by 6-MSITC. These results indicated that 6-MSITC caused cell cycle arrest in G_2/M phase in both HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cells by a *p53*-independent manner.

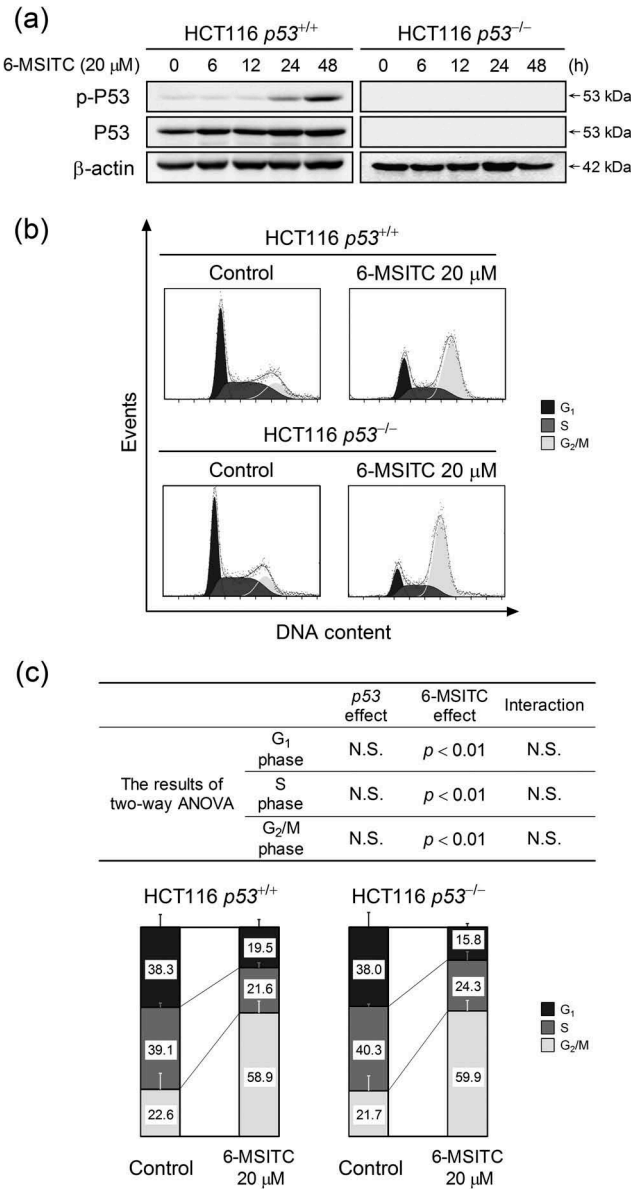


Figure 1. 6-MSITC induces cell cycle arrest in G_2/M phase with *p53* independently.

(a) Phosphorylation and accumulation of P53 protein. Both types of cells were treated with 20 μM 6-MSITC for the indicated times, and the whole cell lysate was used for Western blotting analysis with the indicated specific antibodies. (b) Cell cycle analysis. Both types of cells were treated with 0.2% DMSO as control or 20 μM of 6-MSITC for 24 h. (c) Quantitation of cell cycle distribution in b). The data represent the mean ± SD of combined three independent experiments with duplicate. The results of two-way ANOVA were shown as a table in the upper side of graph (N.S.: not significant).

ERK1/2 activation is recruited for 6-MSITC-caused cell viability inhibition and apoptotic cell death

Accumulated studies reported that MAPKs activated by various stimuli can participate in cell death [16,23]. Thus, we investigated the effects of 6-MSITC on the activation of MAPKs. Interestingly, a time-dependent activation of ERK1/2, but not JNK and p38, was observed in HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cells after treatment with 20 μM 6-MSITC for 6–48 h (Figure 2(a)). Furthermore, MTT assay of the cells treated with

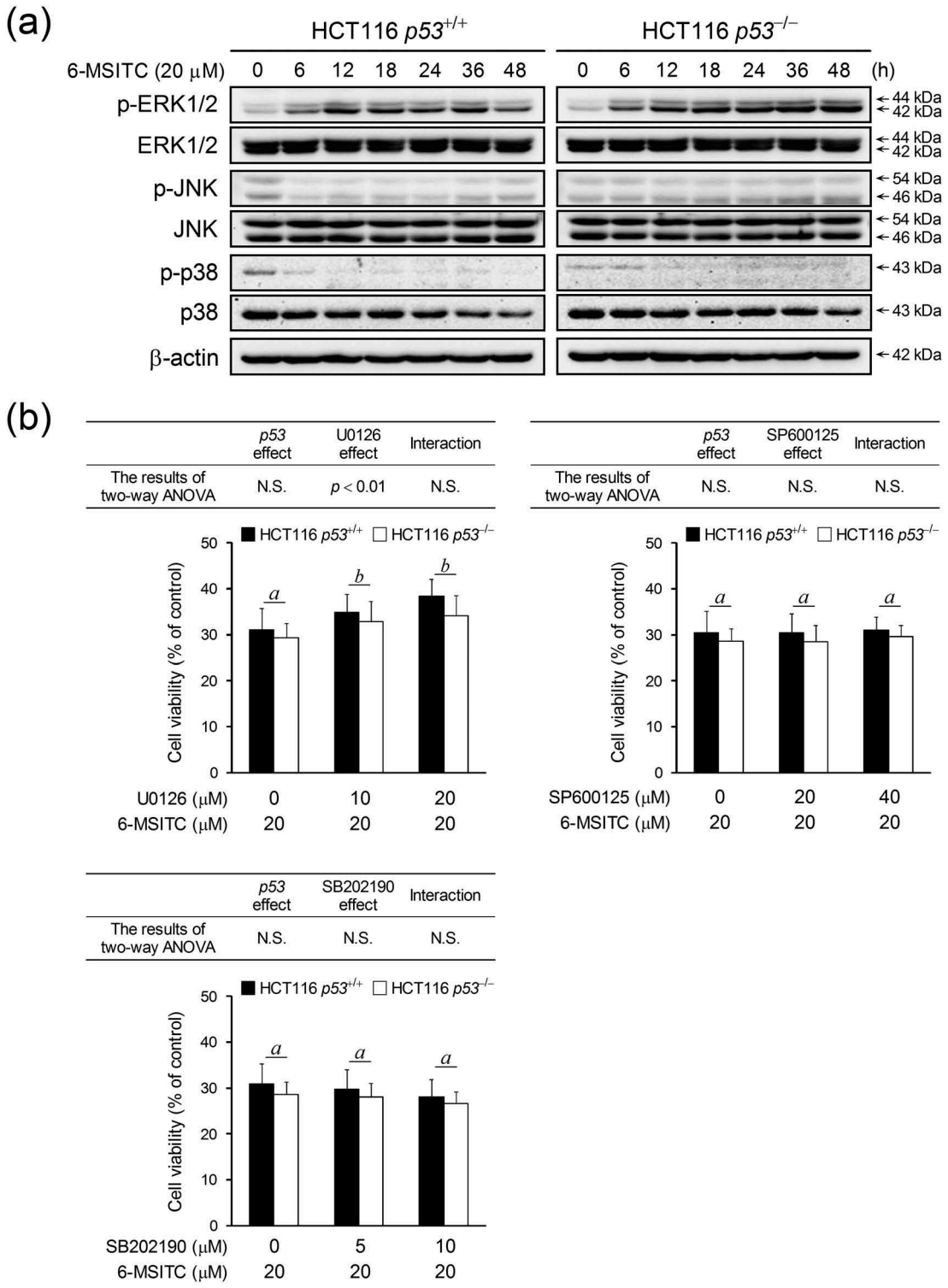


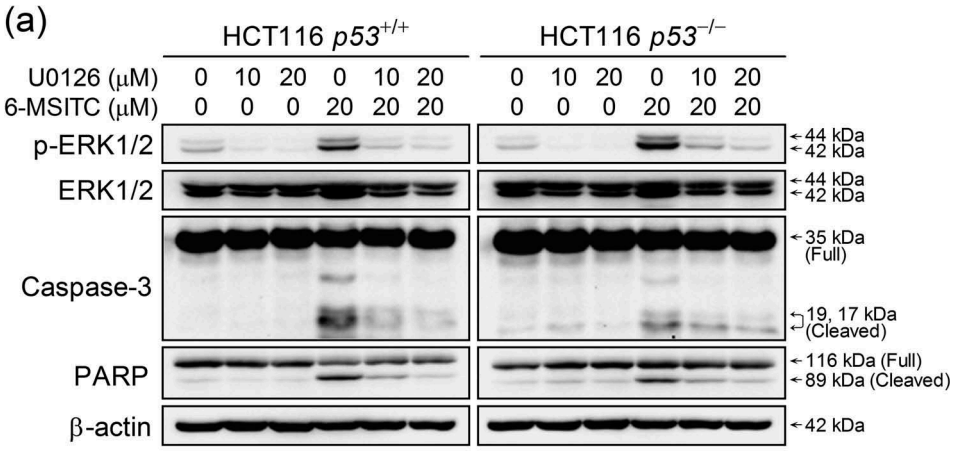
Figure 2. ERK1/2 activation is recruited for 6-MSITC-induced cell death.

(a) Phosphorylation of MAPK. Both types of cells were treated with 20 μM 6-MSITC for the indicated times, and the whole cell lysate was used for Western blotting analysis with the indicated specific antibodies. (b) Cell viability inhibition. Both types of cells were pretreated with the indicated concentrations of MEK1/2 inhibitor (U0126), JNK inhibitor (SP600125) and p38 inhibitor (SB202190) for 2 h and then exposed to 20 μM 6-MSITC for 48 h. The cell viability rate was assessed colorimetrically after staining with MTT, and was expressed as the optical density ratio of the treatment to control. The data represent the mean ± SD of combined three independent experiments with quadruplicate. The results of two-way ANOVA were shown as a table in the upper side of graphs (N.S.: not significant). Different superscript letters indicate significant difference analyzed with Tukey–Kramer test among the groups after two-way ANOVA.

the MAPK inhibitors revealed that MEK1/2 inhibitor (U0126), but not JNK inhibitor (SP600125) and p38 inhibitor (SB202190), enhanced significantly the cell viability that was suppressed by 6-MSITC (Figure 2(b)). As shown in tables of Figure 2(b), two-way ANOVA results indicated that U0126 had significant effect on the inhibition of cell viability in HCT116 cells treated with 6-MSITC while SP600125, SB202190 and *p53* had not such effect. To further elucidate whether the ERK1/2 activation is associated with 6-MSITC-induced apoptosis, we next examined apoptotic events in both types of cells treated with 6-MSITC in the presence or absence of U0126. As shown in Figure 3(a), U0126 blocked 6-MSITC-induced caspase-3 activation and PARP cleavage in both types of cells. U0126 also blocked 6-MSITC-induced apoptotic cell death in both types of cells (Figure 3(b)) in the same fashion. Three-way ANOVA results indicated that U0126, but not *p53*, had significant effect on the apoptosis induction. These results indicated that ERK1/2 activation was recruited for 6-MSITC-induced apoptotic cell death in both HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cells.

6-MSITC activates ERK1/2-mediated ELK1/CHOP/DR5 pathway

ERK1/2 is recently reported to positively regulate DR5 expression by stimulating ELK1 phosphorylation as well as CHOP expression [18–20]. Thus, we investigated the ELK1 phosphorylation and CHOP



(b)

	p53 effect	6-MSITC effect	U0126 effect	Interaction
The results of three-way ANOVA	N.S.	$p < 0.01$	$p < 0.01$	N.S.

	p53 × 6-MSITC	*p53* × U0126	6-MSITC × U0126
The results of two-way interaction	N.S.	N.S.	$p < 0.01$

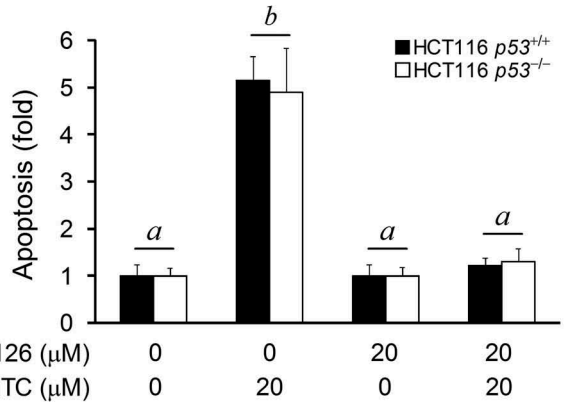


Figure 3. ERK1/2 activation is recruited for 6-MSITC-induced apoptosis.

(a) ERK1/2 activation, caspase-3 activation as well as PARP cleavage. Both types of cells were pretreated with the indicated concentrations of MEK1/2 inhibitor (U0126) for 2 h and then exposed to 20 μM 6-MSITC for 48 h. The whole cell lysate was used for Western blotting analysis with the indicated specific antibodies. (b) Flow cytometric analysis of apoptosis induction. Cell treatment and culture were the same as a). The harvested cells were stained with FITC Annexin V and propidium iodide (PI), followed by flow cytometric analysis. Both FITC Annexin V-positive/PI-negative cells and FITC Annexin V-positive/PI-positive cells were counted as apoptosis, and the fold change of apoptosis was expressed as ratio of the treatment to control. The data represent the mean ± SD of combined four independent experiments with duplicate. The results of three-way ANOVA and two-way interaction were shown as a table in the upper side of graph (N.S.: not significant). Different superscript letters indicate significant difference analyzed with Tukey–Kramer test among the groups after two-way ANOVA (6-MSITC × U0126).

level in both types of cells treated with 20 μM 6-MSITC from 3–48 h. As shown in Figure 4(a), a time-dependent phosphorylation of ELK1 and increase of CHOP level from 6–24 h were observed in both types of cells. Coincident with this, a time-dependent increase of DR5 level and activation of caspase-8 were also observed in both types of cells (Figure 4(b)). Moreover, U0126 blocked phosphorylation of ELK1 and increase of CHOP level in both types of cells treated with 6-MSITC (Figure 5(a)). U0126 also further blocked increase of DR5 level and activation of caspase-8 in both types of cells treated with 6-MSITC (Figure 5(b)). These results suggested that ERK1/2-mediated activation of ELK1 and upregulation of CHOP by 6-MSITC positively regulated DR5 level.

Discussion

6-MSITC is a major bioactive compound in Wasabi, and has been reported to suppress the rat colon carcinogenesis *in vivo* [6] with unclear molecular mechanism. In the present study, we found a novel molecular mechanism for 6-MSITC on the inhibition of the viability of human colorectal cancer cells (HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$).

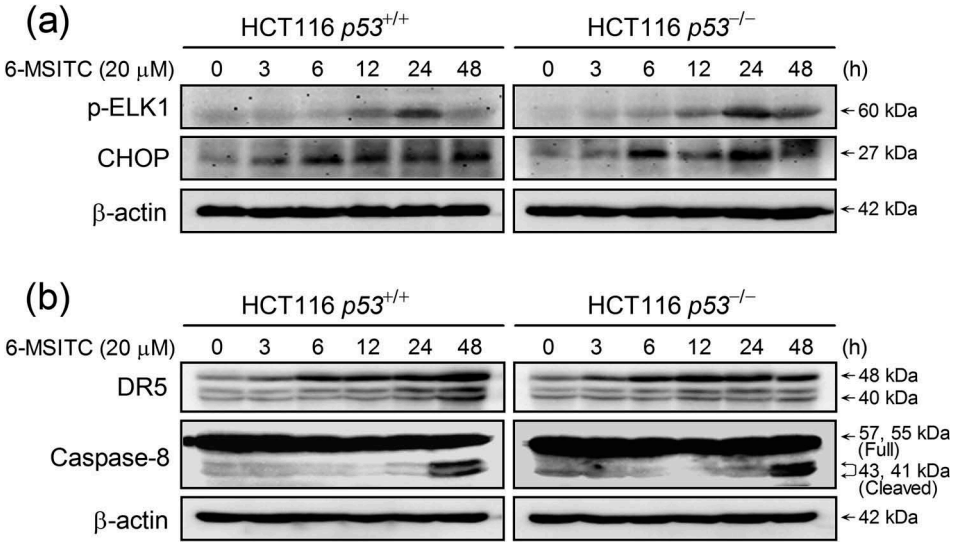


Figure 4. 6-MSITC-activated ERK1/2 positively regulates DR5 level.

(a) and (b) A time-course to detect the phosphorylation of ELK1, levels of CHOP and DR5, and activation of caspase-8. Both types of cells were treated with 20 μM 6-MSITC for the indicated times, and the whole cell lysate was used for Western blotting analysis with the indicated specific antibodies.

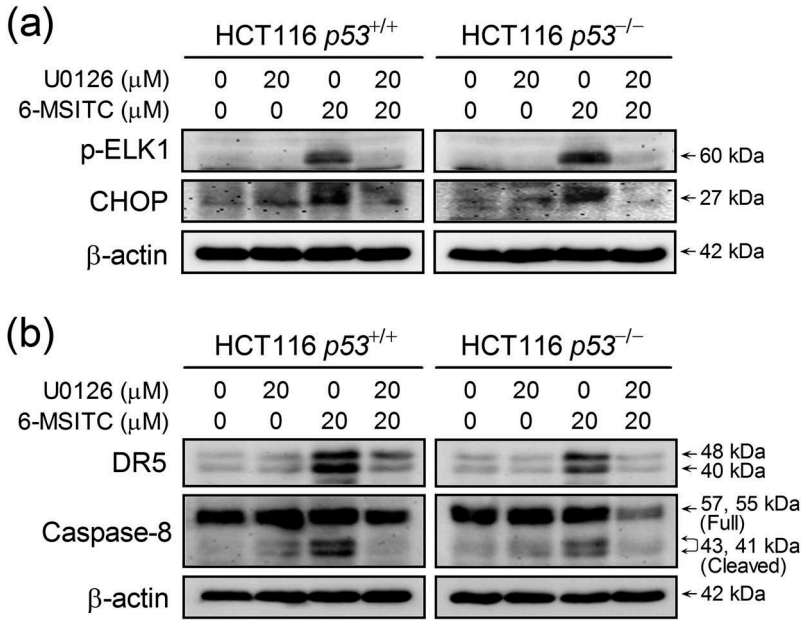


Figure 5. The suppressive effect of U0126 on 6-MSITC-induced activation of ELK1 and CHOP (a), and activation of DR5 and caspase-8 (b).

Both types of cells were pretreated with the indicated concentrations of MEK1/2 inhibitor (U0126) for 2 h and then exposed to 20 μM 6-MSITC for 24 h a) or 48 h b). The whole cell lysate was used for Western blotting analysis with the indicated specific antibodies.

P53, the tumor suppressor protein, plays a central role in cell cycle arrest and apoptosis [13]. Accumulated studies have reported that the loss of normal P53 function due to *p53* gene mutation is observed in a number of human cancers [24,25]. Thus, *p53* mutations attenuate the effects on apoptosis induction by genotoxic reagents [14]. In fact, exposure to genotoxic chemotherapeutic drugs, such as cisplatin, oxaliplatin and 5-fluorouracil, apoptosis induction was observed with much higher level in HCT116 $p53^{+/+}$ cells than that in HCT116 $p53^{-/-}$ cells [26,27]. In the present study, we found that 6-MSITC treatment caused cell cycle arrest in G_2/M phase (Figure 1(b,c)), apoptotic induction and caspase-3 activation (Figure 3(a,b)) in both types of cells as the similar actions. Although a light increase in the phosphorylation and accumulation of P53 protein was observed in HCT116 $p53^{+/+}$ cells (Figure 1(a)) 24 h-late after treatment with 6-MSITC, in which, the apoptosis was already beginning. Many chemotherapeutic drugs are known to exert their anti-cancer effects by activating P53 with genotoxic stress, which resulted in side effect and caused toxicity to normal cells [14]. It is also reported that chemotherapeutic drugs treatment increased highly P53 protein level at early time (8–12 h) in HCT116 $p53^{+/+}$ cells [28,29]. On the other hand, lately increased P53 level is reported to contribute to the clearance of death cells by macrophages engulfment [30]. In this study, we also observed the light phosphorylation of P53 in HCT116 $p53^{+/+}$ cells (Figure 1(a)) at late stage (24–48 h), but not at early stage, by 6-MSITC. Other studies have reported that phenethyl isothiocyanate (PEITC) induced apoptosis in *p53*-deficient human prostate cancer cells [31], and benzyl isothiocyanate (BITC) induced apoptosis in human breast cancer cells silenced by P53 siRNA [32]. In addition, sulforaphane, an analogues of 6-MSITC, has been reported to selectively induce apoptosis in HCT116 cells, but not normal colon mucosal epithelial cell [33]. These data suggested that apoptotic induction by 6-MSITC is not due to P53 activation, and is different with that by other genotoxic chemotherapeutic drugs in human colon cancer cells.

To clarify the upstream signaling of 6-MSITC-induced apoptosis, we further investigated the effects of 6-MSITC on MAPKs signaling which is demonstrated to participate in multiple cellular events including apoptosis induction [16,23]. Interestingly, 6-MSITC induced the phosphorylation of ERK1/2, but not JNK and p38, in both types of cells (Figure 2(a)). MEK1/2 inhibitor (U0126), but not JNK inhibitor (SP600125) and p38 inhibitor (SB202190), enhanced significantly the cell viability that was suppressed by 6-MSITC (Figure 2(b)). U0126 further blocked 6-MSITC-induced apoptotic cell death, caspase-3 activation and PARP cleavage (Figure 3). These data suggested that ERK1/2 activation is recruited for 6-MSITC-induced apoptosis. To future confirm the effect of this overexpression system on cell death-induced by 6-MSITC, the constructs of active ERK1/2 and/or inactive variants will be conducted in our next research.

Recent studies have reported that activation of ELK1 and increase of CHOP level through ERK1/2 positively regulated DR5 expression by directly binding to *dr5* gene as enhancer [18–20]. Knock down of ERK1/2 decreased ELK1 phosphorylation and CHOP level [19]. In the present study, we found that 6-MSITC enhanced ELK1 phosphorylation and CHOP level, and finally increased DR5 level in both types of cells (Figure 4(a,b)). Furthermore, U0126 inhibited both phosphorylation of ELK1 and increase of CHOP level, consequently downregulated DR5 level in both types of cells treated with 6-MSITC (Figure 5(a,b)). These results suggested that activation of ERK1/2 by 6-MSITC upregulated DR5 level without *p53*-dependence, leading to apoptotic cell death. Several lines of studies have reported that various cellular stress, such as DNA damage, oxidative stress and endoplasmic reticulum stress, induced CHOP expression in a *p53*-independent manner [34,35]. Accumulated data have shown that CHOP expression is mainly regulated at the transcriptional level [36]. In addition, expression of CHOP is also regulated at post-transcriptional level, such as mRNA stability [36]. In fact, MEK1/2 inhibitor such as U0126 has been reported to suppress mRNA expression level of CHOP in HCT116 cells [37]. On the other hand, recent studies have shown that DR5 protein might be degraded through deubiquitination [38,39]. In our previous study, we found that 6-MSITC could upregulate the levels of NRF2 by inhibiting the ubiquitination and proteasomal turnover of NRF2 [40]. The effect of 6-MSITC on upregulatinging the levels of DR5, CHOP need to clarify possible proceed via transcriptional or post-transcriptional or post-translational level, which need to be clarified in our next work. Therefore, 6-MSITC seems to be a potential candidate of DR5 inducer for colon cancer chemoprevention and chemotherapy.

DR5 is a member of TNF receptor superfamily and cytoplasmic death domain-containing cell surface protein [41]. Increase of DR5 expression by various stimuli, such as small molecule anti-cancer drugs and bioactive compounds, triggers extrinsic apoptotic pathway, ultimately leading to apoptotic cell death [42]. Furthermore, *dr5* gene mutation was detected at very low frequency in various cancers [42,43]. Hence, increase of DR5 expression by bioactive reagents has been recognized as effective cancer therapeutic approach to sensitize effect of combination with TRAIL [42]. Although P53 plays a key role in expression of DR5 [44], DR5 has been also reported to be regulated in a *p53*-independent manner [45]. For instance, celecoxib, a nonsteroidal anti-inflammatory drugs (NSAIDs), increased DR5 expression and sensitized the effect of TRAIL-induced apoptosis to colon cancer cells, with *p53* independence [46].

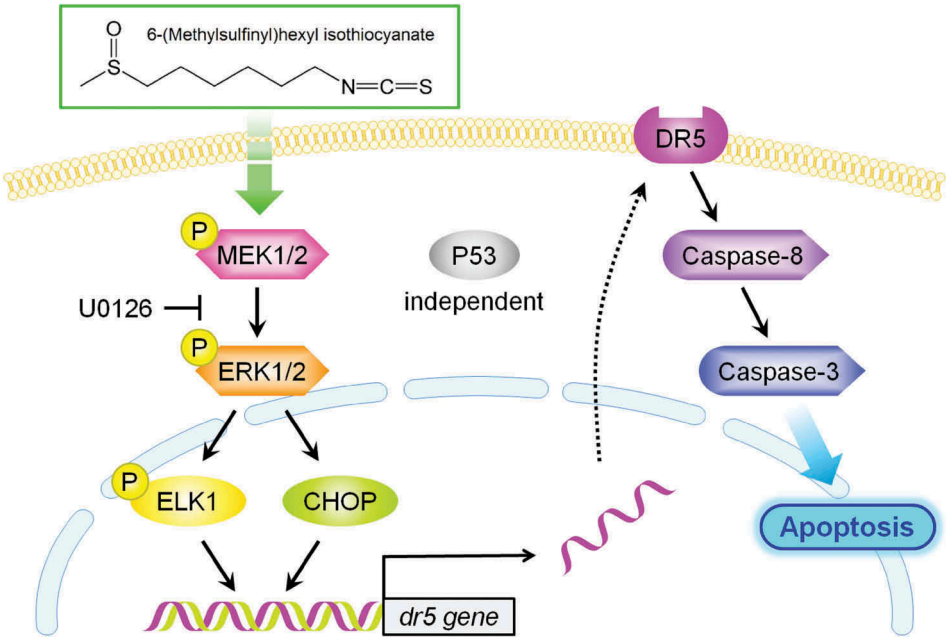


Figure 6. Involvement of ERK1/2-mediated ELK1/CHOP/DR5 pathway in 6-MSITC-induced apoptosis.

Based on our present and previous data, we found that 6-MSITC targeted ERK1/2-mediated ELK1/CHOP/DR5 pathway in a time series. 6-MSITC caused ERK1/2 phosphorylation from 6 h, ELK1 phosphorylation and CHOP expression from 6 h, caspase-8 and caspase-3 activation from 24–36 h [47]. Therefore, we considered that ERK1/2-mediated ELK1/CHOP/DR5 pathway is involved in 6-MSITC-induced apoptosis in HCT116 cells although the direct evidence by knocking out the key factors in this pathway is required in future research.

Although our data showed that 6-MSITC induced apoptosis in HCT116 cells involved ERK1/2-mediated ELK1/CHOP/DR5 pathway, rather than *p53* pathway. We also noted that the mechanisms for 6-MSITC-induced apoptosis may be complicated and multiple. For example, 6-MSITC also lightly reduced the phosphorylation of JNK and p38 after 6 h of treatment. It is reported that p38 activation is required for cancer cell survival in colon cancer [48], and activation of JNK promotes colon carcinogenesis [49]. Thus, it is possible that 6-MSITC also inhibited cell survival of both HCT116 $p53^{+/+}$ and $p53^{-/-}$ cells by partially inhibiting p38 and JNK activation. On the other hand, how 6-MSITC activated ERK1/2 is still unclear. It has been reported that RAS membrane association as posttranslational modifications lead to sustain the ERK1/2 activation [50]. Moreover, early growth response 1 (EGR1) can upregulate the expression of geranylgeranyl diphosphate synthase (GGPPS), which catalyzes the synthesis of GGPP to increase RAS prenylation and membrane association [51,52]. Sulforaphane, an analogues of 6-MSITC, has reported to increase EGR1 level to induce cell cycle arrest and apoptosis, and suppress tumorigenesis in mouse xenograft model [53]. In our primary investigation, we also observed that 6-MSITC increased EGR1 level in both types of cells (data not shown). Therefore, it is possible that 6-MSITC activates ERK1/2 via upregulating EGR1 expression, which needs to be investigated in our future work.

In summary, we demonstrated that 6-MSITC induced cell cycle arrest and apoptosis in both HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cells in the same effect. Molecular data revealed that the activation of ERK1/2, rather than *p53*, is recruited for 6-MSITC-induced apoptotic cell death and suggested that ERK1/2-mediated ELK1/CHOP/DR5 pathway is involved in the molecular mechanisms (Figure 6). These findings will help us to understand the chemoprevention mechanisms of 6-MSITC on colon carcinogenesis previously reported in animal model.

Acknowledgments

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Authors' contribution

S.Y. and D.-X.H. designed the experiments. S.Y. performed the experiments. S.Y., S.W. and K.S. analyzed the data. S. Y. and D.-X.H. wrote the paper. D.-X.H. administrated this research.

Disclosure statement

No potential conflict of interest was reported by the authors.

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