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8-Methoxypsoralen Induces Apoptosis by Upregulating p53 and Inhibits Metastasis by Downregulating MMP-2 and MMP-9 in Human Gastric Cancer Cells

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Abstract

Furanocoumarin 8-methoxypsoralen (8-MOP) is the parent compound that naturally occurs in traditional medicinal plants used historically. 8-MOP has been employed as a photochemotherapeutic component of Psoralen + Ultraviolet A (PUVA) therapy for the treatment of vitiligo and psoriasis. Although the role of 8-MOP in PUVA therapy has been studied, little is known about the effects of 8-MOP alone on human gastric cancer cells. In this study, we observed anti-proliferative effect of 8-MOP in several human cancer cell lines. Among these, the human gastric cancer cell line SNU1 is the most sensitive to 8-MOP. 8-MOP treated SNU1 cells showed G1-arrest by upregulating p53 and apoptosis by activating caspase-3 in a dose-dependent manner, which was confirmed by loss-of-function analysis through the knockdown of p53-siRNA and inhibition of apoptosis by Z-VAD-FMK. Moreover, 8-MOP-induced apoptosis is not associated with autophagy or necrosis. The signaling pathway responsible for the effect of 8-MOP on SNU1 cells was confirmed to be related to phosphorylated PI3K, ERK2, and STAT3. In contrast, 8-MOP treatment decreased the expression of the typical metastasis-related proteins MMP-2, MMP-9, and Snail in a p53-independent manner. In accordance with the serendipitous findings, treatment with 8-MOP decreased the wound healing, migration, and invasion ability of cells in a dose-dependent manner. In addition, combination treatment with 8-MOP and gemcitabine was effective at the lowest concentrations. Overall, our findings indicate that oral 8-MOP has the potential to treat early human gastric cancer, with fewer side effects.

Key Words: 8-Methoxypsoralen, Apoptosis, Gastric cancer, Metastasis, MMP-2, p53

INTRODUCTION

Psoralen is the parent compound in a family of furanocoumarins and naturally occurs in traditional medicinal plants such as *Psoralea corylifolia*, *Ficus carica*, and *Ammi majus*, whose juice and fruit have been used for skin tanning in the historical period (Pathak and Fitzpatrick, 1992). Since the 1970s, psoralen tablets made of 8-methoxypsoralen (8-MOP) have been used as photochemotherapeutic drugs for the treatment of various dermatologic proliferative disorders, including vitiligo, psoriasis, and cutaneous T-cell lymphoma (Parrish *et al*., 1974; Wolff *et al*., 1976; Melski *et al*., 1977; Henseler *et al*., 1981). For Psoralen+Ultraviolet A (PUVA) therapy, psoralen (P) is administered together with exposure to ultraviolet A (UVA) radiation; hence, the term PUVA. According to the mechanism of action of PUVA therapy reported previously (Hearst *et al*., 1984), the planar structure of 8-MOP is activated by UVA irradiation to form pyrimidine compounds within cells and further photochemically reacts with pyrimidine bases of DNA to induce interstrand crosslinking. *In vitro* interaction of 8-MOP with DNA in darkness revealed that 8-MOP binds to DNA for inducing compaction without severely disturbing DNA structures (Arabzadeh *et al*., 2002), requiring complementary exposure to UVA for DNA fragmentation (Liu *et al*., 1999). In contrast, the symptoms of PUVA therapy have extended from dermatological disorders to diverse disorders, including bovine proliferative vascular smooth muscle cells (March *et al*., 1993) and human breast cancer cells (Panno *et al*., 2009). Many effectors and signaling pathways have been studied to characterize the mechanisms of action of PUVA therapy (Canton

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et al., 2002; Santamaria *et al*., 2002; Bartosová *et al*., 2006; Panno *et al*., 2009; Stadler *et al*., 2009; Xia *et al*., 2014). Previous studies have characterized the mechanisms underlying PUVA therapy and are associated with cell death caused by DNA damage, cell cycle arrest, mitochondrial dysfunction, etc. The major mechanism of action of PUVA therapy is mediated by the upregulation of p53, which in turn induces apoptosis via the upregulation of p21 (Santamaria *et al*., 2002; Panno *et al*., 2009). However, the concept of PUVA therapy has changed since 8-MOP alone has been proven to decrease the viability of human liver cancer cells, HepG2, regardless of UV exposure (Peng *et al*., 2012).

It is well known that p53 protein is a major tumor suppressor in diverse cancers, playing the role of a cell-cycle checkpoint to maintain genetic stability (Vogelstein *et al*., 2000). It also plays a critical role in gastric cancer (Imazeki *et al*., 1992; Gabbert *et al*., 1995; Vogelstein *et al*., 2000; Fenoglio-Preiser *et al*., 2003; Busuttil *et al*., 2014). For example, p53 expression is inhibited in premalignant intestinal metaplasia lesions in gastric cancer (Busuttil *et al*., 2014). Approximately 50% of gastric cancer patients exhibit genetic and epigenetic changes that cause p53 inactivation (Imazeki *et al*., 1992; Gabbert *et al*., 1995). By contrast, targeting the malignant invasion of cancer cells is important for overcoming cancer. The matrix metalloproteinase (MMP) family comprises dozens of zinc-dependent endopeptidases involved in extracellular matrix degradation that are important for cancer progression, cell adhesion, migration, invasion, and angiogenesis (Gialeli *et al*., 2011). Given their unique ability to degrade major components of the basement membrane, gelatinases MMP-2 and MMP-9 are the most important MMPs involved in tumor invasion and metastasis (Talvensaari-Mattila *et al*., 1998; Li *et al*., 2004).

Although many aspects of PUVA therapy have been extensively studied, the effects of 8-MOP on human gastric cancer cells have not been elucidated. In this study, we found for the first time that 8-MOP alone inhibited the viability of the human gastric cancer cell line SNU1 via p53 upregulation and caspase-3 activation. Furthermore, 8-MOP reduces cell mobility to inhibit metastasis in a p53-independent manner. Overall, these results raise the possibility of 8-MOP becoming a part of combination chemotherapy for gastric cancer, in addition to other well-known drugs.

MATERIALS AND METHODS

Reagents and human cancer cell lines

General chemicals and reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise. Gemcitabine and 8-MOP were purchased from Sigma-Aldrich. Inhibitors including Z-VAD-FMK, 3-MA, and necrostatin-1 were purchased from Selleckchem (Houston, TX, USA).

All human cancer cell lines were obtained from the Korean Cell Line Bank (KCLB, Seoul, Korea). Two cell lines, if available, were used for each type of cancer. The human cancer cell lines used in this study were as follows: A549 and H1650 (lung cancer); SNU449 and SK-Hep1 (hepatocellular carcinoma); MDA-MB453 and T47D (breast cancer); SKOV3 (ovarian cancer); PC3 and DU145 (prostate cancer); HCT15 and DLD1 (colorectal cancer); AGS and SNU1 (gastric cancer harboring wild-type p53); and SK-MEL-2 (skin cancer). Cells were cultivated in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% fetal bovine serum (FBS) (Thermo-Fisher Scientific, Waltham, MA, USA) and 1% antibiotic-antimycotic solution at 37°C in 5% CO_2 atmosphere, unless stated otherwise.

Proliferation assay

The cytotoxicity of 8-MOP against SNU1 cells was determined using the Cell Counting Kit-8 (CCK-8) assay kit (Sigma-Aldrich) according to the manufacturer's instructions. Cells were seeded in 96-well plates and incubated for 18 h, followed by 8-MOP treatment at 2-fold increasing concentrations up to 250 μM for 48 h. Following treatment, the cells were incubated with 20 μL of CCK-8 solution for 1-2 h, and absorbance was measured at 460 nm using a microplate reader (SpectraMax-i3x; Molecular Devices, San Jose, CA, USA).

Flow cytometry

Cells (1×10^6) were treated with 100, 200, or 300 μM of 8-MOP for 24 h and then treated with trypsin. The detached cells were fixed and permeabilized with 70% methanol at –20°C overnight. After washing with ice-cold phosphate-buffered saline, the cells were stained with DAPI (4',6-Diamidino-2-Phenylindole, Thermo-Fisher Scientific for cell cycle phase) or AnnexinV-PI (propidium iodide) (Annexin V apoptosis detection kit, Thermo-Fisher Scientific for apoptosis) in the dark for 10 min at room temperature, according to the manufacturer's instructions. Flow cytometry was performed using Attune CytPix (Thermo-Fisher Scientific). Ten thousand events per sample were counted in duplicate.

Western blot analysis

Equal amounts of proteins were loaded on 10-15% SDS-PAGE gels, and the separated bands were transferred onto polyvinylidene fluoride (PVDF) membranes. Membranes were blocked with 5% non-fat milk in Tris-buffered saline and 0.1% Tween-20 buffer. Antibodies used in western blot analysis were purchased from Santa Cruz Biotechnology (Dallas, TX, USA), unless stated otherwise. The blotted membrane was then incubated with antibodies against cleaved caspase-3, poly(ADP-ribose) polymerase (PARP), MMP-2, MMP-9, Snail (Cell Signaling Technology, Danvers, MA, USA), β-actin, and p53 at 4°C overnight. After washing, the blots were incubated with horseradish peroxidase-labeled secondary antibodies at room temperature for 2 h. Bands were visualized by enhanced chemiluminescence using LuminiGraph I (ATTO, Tokyo, Japan).

RNA interference

Cells were transiently transfected with scrambled (sc)- or p53-siRNA using Lipofectamine RNAi MAX reagent (Thermo-Fisher Scientific/Invitrogen) according to the manufacturer's protocol. Transfected cells were cultivated in the presence or absence of 200 μM 8-MOP for 48 h. The cells were stained with DAPI and subjected to flow cytometry. The p53-siRNA sequence used in this study was 5′-ACUCCACACGCAAAUUUCCTT-3′.

Wound healing assay

SNU1 cells were seeded in 6-well plates at a density of 5×10^5 cells/well to obtain a confluent monolayer. After overnight incubation, a straight line was scratched in the middle of the monolayer using a pipette tip. The cells were washed with

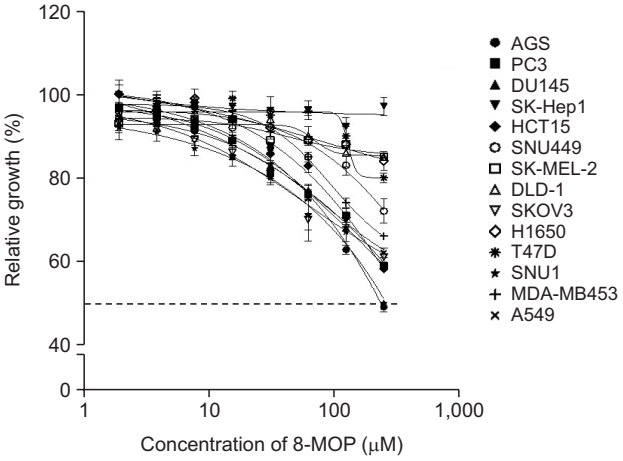

Fig. 1. Viability test of human cancer cell lines against 8-MOP. The viability of 14 human cancer cell lines was measured by CCK-8 assay. Each cell line was cultured and treated with the indicated concentrations of 8-MOP up to 250 μM. Cell viability was measured after 48 h and analyzed using GraphPad Prism 5.0. The human cancer cell lines used are as follows: A549 and H1650 (lung cancer); SNU449 and SK-Hep1 (hepatocellular carcinoma); MDA-MB453 and T47D (breast cancer); SKOV3 (ovarian cancer); PC3 and DU145 (prostate cancer); HCT15 and DLD1 (colorectal cancer); AGS and SNU1 (gastric cancer); and SK-MEL-2 (skin cancer). *n*=3.

1 mL PBS to remove cellular debris, placed in 2 mL medium, and treated with 100, 200, or 300 μM 8-MOP. After 24 h of incubation, the width of the scratched wound was measured and compared to that observed at 0 h. All experiments were carried out in triplicate and repeated three times.

Migration assay

The migration ability of SNU1 cells was measured using Transwell chambers with 8 μm filter inserts (Corning Inc., Corning, NY, USA) without Matrigel (Corning Inc.). Cells were seeded in inserts (1×10^3 cells/insert) and treated with 100, 200, or 300 μM 8-MOP in RPMI-1640 medium without FBS. After incubation for 24 h, the inserts were fixed with 500 μL of 4% paraformaldehyde for 15 min and stained with a Brilliant Blue solution for 15 min. The inserts were washed several times with distilled water and the migrated cells were counted from an average of five random visual fields under a microscope. All experiments were performed in triplicates and repeated three times.

Invasion assay

SNU1 cells were seeded in inserts (1×10^3 cells/insert) in a Transwell chamber (Corning Inc.) and treated with 100, 200, or 300 μM 8-MOP in RPMI-1640 medium without FBS. The outer membrane of a polycarbonate membrane insert (pore size 8 μm) was coated with 0.5 mg/mL collagen, and the insert was filled with 10 μL Matrigel diluted with 40 μL PBS. After incubation for 24 h at 37°C, the inserts were fixed with 500 μL of 4% paraformaldehyde for 15 min and stained with Brilliant Blue solution for 15 min. The insert was washed several times with distilled water and the migrated cells were counted from an average of five random visual fields under a microscope. All experiments were performed in triplicate and repeated three times.

Statistical analysis

All experiments were performed in triplicate and repeated at least three times. Data are represented as mean ± SD, unless indicated otherwise. Analysis of variance with Tukey's test was performed for multiple comparisons among groups using GraphPad Prism 5.0 software (GraphPad, La Jolla, CA, USA). Results with *p*-values <0.05 were considered statistically significant.

RESULTS

8-MOP inhibits the viability of gastric cancer cell lines

Following treatment with 8-MOP alone, we observed changes, in a dose-dependent manner, in the viability of diverse human cancer cell lines including lung, liver, breast, ovarian, prostate, colorectal, skin, and gastric cancers. As shown in Fig. 1, among the 14 cancer cell lines only a couple of gastric cancer cell lines showed detectable IC_{50} values (dotted line), when treated up to the 8-MOP concentration of 250 μM, as the follows: AGS cells, 280.1 μM; and SNU1 cells, 222.5 μM. In contrast, the remaining cell lines did not show detectable IC_{50} values, although some showed a slight decrease in their viability.

The IC_{50} analysis revealed, for the first time, that 8-MOP treatment alone, without UVA irradiation, inhibits the viability of human gastric cancer cells. These results prompted us to elucidate the mechanism underlying the anti-proliferative effects of 8-MOP in SNU1 cells, the most 8-MOP sensitive gastric cancer cell line.

8-MOP induces G1-arrest via p53 upregulation and apoptosis via caspase-3 activation

As the first step in determining the anti-proliferative effects of 8-MOP in SNU1 cells, the percentile change of G1-arrest or apoptosis in response to treatment with 8-MOP was analyzed by flow cytometry following DAPI or annexin V/PI staining, respectively. As shown in Fig. 2A, the population of cells in the sub-G1 phase increased significantly in a dose-dependent manner following treatment with 8-MOP for 24 h, compared to the untreated cells. When treated at 100 μM 8-MOP, 17.1% of the cells remained in the sub-G1 phase; at 200 μM 8-MOP, 24.8%, and at 300 μM 8-MOP, 35.9%. Similarly, a profound increase ($p<0.01$) in the population of late apoptotic cells was observed along with a slight increase in the proportion of cells in early apoptosis in a dose-dependent manner (Fig. 2B). Treatment with 8-MOP concentrations of 100 μM, 200 μM, and 300 μM showed an early apoptosis: late apoptosis ratios of 3.2:17.0, 4.6:32.9, and 4.9:51, respectively.

Next, to confirm the underlying mechanism of 8-MOP-induced G1-arrest and apoptosis in SNU1 cells, western blot analysis was conducted using antibodies against p53, a canonical G1-arrest marker, and against PARP and cleaved caspase-3, typical apoptotic markers. As expected, we found that exposure of gastric cancer cell lines to 8-MOP resulted in a significant increase in the cellular levels of p53, PARP, and cleaved caspase-3 in a dose-dependent manner, compared to that in untreated cells (Fig. 2C). These results suggested that 8-MOP-induced G1-arrest and apoptosis in SNU1 cells were caused by the upregulation of p53 and caspase-3 via PARP,

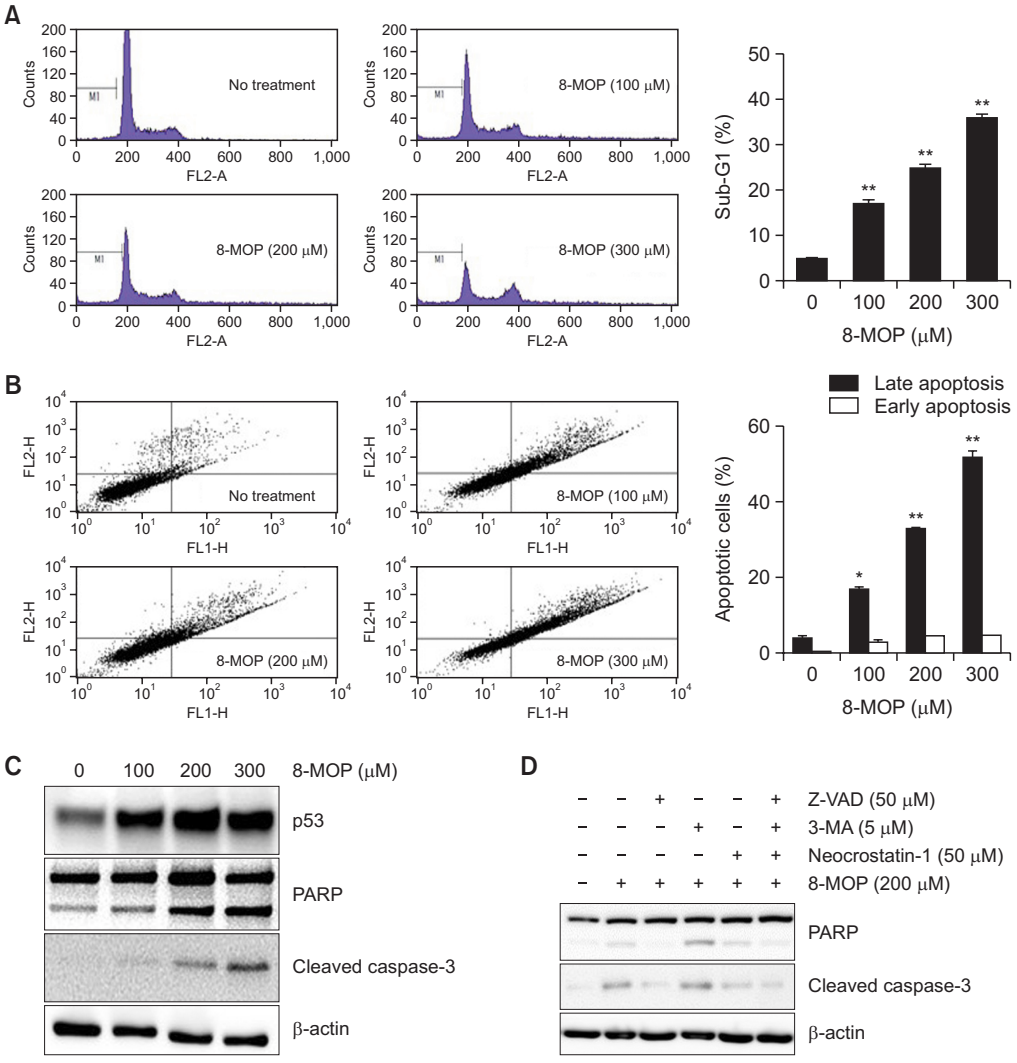

Fig. 2. 8-MOP-induced G1-arrest and apoptosis in a dose-dependent manner. (A) Dose-dependent G1-arrest by 8-MOP. SNU1 cells were treated with 8-MOP concentrations of 100, 200, and 300 μM for 24 h and subjected to flow cytometry analysis after DAPI staining to analyze the cell cycle phases. Cell populations of G1-arrest were analyzed, and the FACS data were visualized into graphs using GraphPad Prism 5.0, compared with the untreated control. *n*=3; ***p*<0.01, *vs* untreated controls. (B) Dose-dependent apoptosis was induced by treatment with 8-MOP. SNU1 cells were treated with 8-MOP concentrations of 100, 200, and 300 μM for 24 h and subjected to flow cytometry analysis after annexin V-PI staining to analyze apoptosis. Cell populations of early or late apoptosis were analyzed, and the FACS data were visualized in graphs using GraphPad Prism 5.0, compared with the untreated control. *n*=3; **p*<0.05, ***p*<0.01, *vs* untreated controls. (C) Induction of apoptosis via p53 upregulation and caspase-3 activation. SNU1 cells were treated with 8-MOP concentrations of 100, 200, and 300 μM for 24 h and subjected to western blot analysis using the indicated antibodies against p53, PARP, or cleaved caspase-3, compared with untreated control. β-actin was used as a normalization control. (D) 8-MOP-induced apoptosis, but not autophagy or necrosis. SNU1 cells were treated with 8-MOP concentrations of 200 μM for 24 h in the presence or absence of the pan-caspase inhibitor (50 μM Z-VAD-FMK), autophagy inhibitor (5 mM 3-MA), or necrosis inhibitor (50 μM necrostatin-1) and subjected to western blot analysis using the indicated antibodies against PARP or cleaved caspase-3. β-actin was used as a normalization control.

respectively. For further in-depth study, we determined whether 8-MOP-induced apoptosis was attributed to caspase-3, autophagy, or necrosis using inhibitors such as Z-VAD-FMK (pan-caspase inhibitor), 3-MA (autophagy inhibitor), and necrostatin-1 (necrosis inhibitor). As shown in Fig. 2D, pretreatment with 50 μM Z-VAD-FMK almost abrogated the activation of procaspase-3 and PARP induced by treatment with 200 μM 8-MOP in SNU1 cells. In contrast, pretreatment with 5 mM 3-MA or 50 μM necrostatin-1 had no effect. These results indicate that 8-MOP induces caspase-dependent apoptosis in the gastric cancer cell line SNU1.

The 8-MOP-induced G1-arrest is abrogated by loss-of-function of p53 and caspase

The above results suggest that 8-MOP-induced G1-arrest and apoptosis are attributed to gain-of-function, such as p53 upregulation and activation of caspase-3, respectively. For further in-depth study, these results were validated through the concept of loss-of-function using specific siRNAs and inhibitors. In this regard, the p53-dependent G1-arrest and cas-

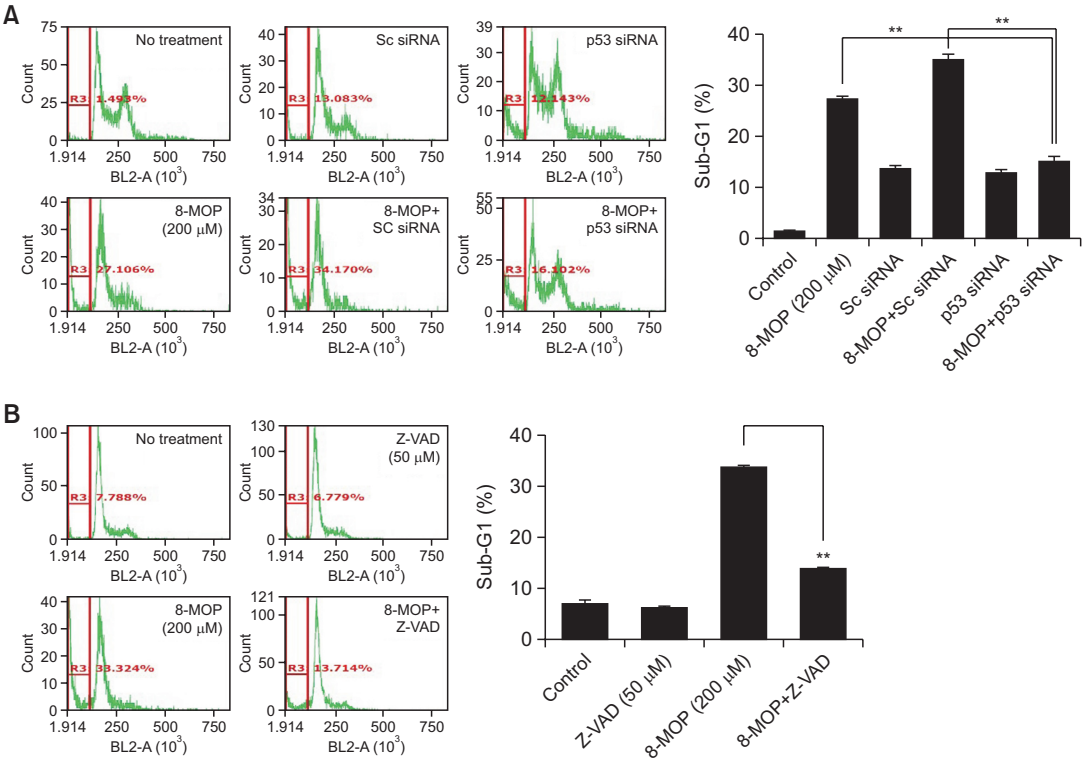

Fig. 3. Loss-of-function analysis of on the 8-MOP-induced G1-arrest and apoptosis. (A) Loss-of-function analysis of the 8-MOP-induced G1-arrest using p53-siRNA. SNU1 cells were transfected with scrambled(sc)-siRNA or p53-siRNA. The cells were incubated in the presence or absence of 8-MOP concentration of 200 μM for 48 h and subject to flow cytometry analysis. Populations of G1-arrest were analyzed, and the FACS data was visualized into graph using GraphPad Prism 5.0, compared with controls. *n*=3; ***p*<0.01 *vs* 8-MOP-treated alone or control treated with 8-MOP and sc-siRNA. (B) Loss-of-function analysis of 8-MOP-induced apoptosis using Z-VAD-FMK. SNU1 cells were transfected with or without 50 μM of Z-VAD-FMK. The cells were incubated in the presence or absence of 8-MOP concentration of 200 μM for 48 h and subject to flow cytometry analysis. Populations of apoptosis were analyzed, and the FACS data was visualized into graph using GraphPad Prism 5.0, compared with control. *n*=3; ***p*<0.01 *vs* control treated with 8-MOP alone.

pase-dependent apoptosis in response to 8-MOP treatment were confirmed using p53-siRNA and the apoptosis inhibitor, Z-VAD-FMK. Compared with no significant effects of sc-siRNA on G1-arrest, pretreatment with p53-siRNA significantly abrogated the 200 μM 8-MOP-induced G1-arrest in SNU1 cells via p53 knockdown (Fig. 3A). Similarly, pretreatment with 50 μM Z-VAD-FMK, an apoptosis inhibitor, significantly abrogated the 200 μM 8-MOP-induced G1-arrest in SNU1 cells (Fig. 3B). Consequently, 8-MOP-induced apoptosis did not occur after p53-siRNA pre-treatment, as determined by FACS analysis (data not shown). These results confirm that 8-MOP-induced G1-arrest followed by apoptosis is attributed to the upregulation of p53.

8-MOP inhibits expression of metastasis-related proteins in a p53-independent manner

To identify effector proteins involved in 8-MOP-induced apoptosis, we screened the expression changes of several candidates using an immune blotting assay. We found that the expression of metastasis-related proteins, including MMP-2, MMP-9, and Snail, was inhibited despite upregulating the expression of p53 in response to 8-MOP treatment (Fig. 4A, upper part). The signaling pathways involved in 8-MOP-induced apoptosis and/or downregulation of metastasis-related proteins were attributed to the inactivation of well-known signaling MAP kinases, including PI3K, ERK2, and STAT3 (Fig. 4A, lower part). As 8-MOP inhibited the expression of metastasis-related proteins while increasing the expression of p53, we examined the relationship between p53 and metastasis-related proteins. As shown in Fig. 4B, the expression of metastasis-related proteins was only affected by the presence of 8-MOP, irrespective of expression of p53. These results suggest that the expression of metastasis-related proteins is not associated with the expression of p53.

8-MOP decreases the cell mobility of wound healing, migration, and invasion in a dose-dependent manner

These results prompted us to examine whether 8-MOP affects cell motility, including wound healing, invasion, and migration. As shown in Fig. 5, upper part, treatment with 8-MOP affected wound healing, migration, and invasion abilities in a dose-dependent manner compared to untreated controls. In detail (Fig. 5, lower part), the wound healing rates after 24-h of treatment with 8-MOP concentrations of 100, 200, and 300 μM decreased to 94.7%, 54.3%, and 44.6%, respectively, when compared to those of controls. Cell populations migrating through the membrane in the group after 24-h treatment with 8-MOP concentrations of 100, 200, and 300 μM decreased to 68.5%, 82.3%, and 96.5%, respectively. Similarly, the percentage of invasive cells passing through Matrigel in the group

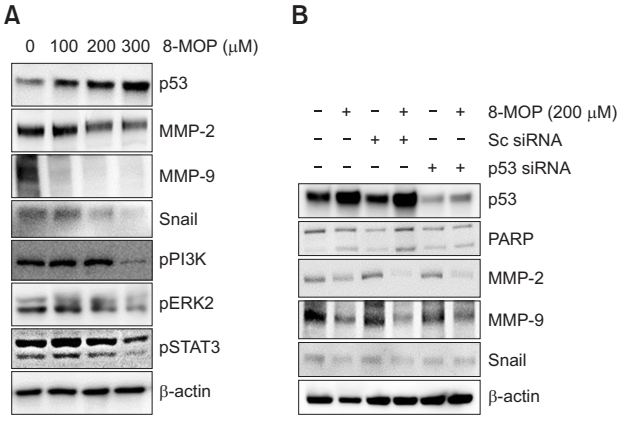

Fig. 4. Immunoblot analysis of signaling proteins for the 8-MOP-induced apoptosis. (A) Immunoblot analysis of signaling proteins. Immunoblot analysis was performed to investigate intracellular signaling proteins responsible for the 8-MOP induced G1-arrest and apoptosis. SNU1 cells were treated with 8-MOP concentrations of 100, 200, or 300 μM for 24 h and subject to immunoblot analysis to detect the expression of the signaling molecules including p53, MMP-2, MMP-9, Snail, pPI3K, pERK2, and pSTAT3, compared with untreated control. β-actin was used as a normalization control. Note that the typical metastasis-related proteins including MMP-2, MMP-9, and Snail were found to be reduced by 8-MOP treatment. (B) Effects of p53-siRNA treatment on the signaling molecules. Immunoblot analysis was performed to investigate whether the 8-MOP-reduced metastasis proteins is associated with p53 expression. SNU1 cells were treated with or without 200 μM of 8-MOP for 24 h and transfected with sc-siRNA or p53-siRNA. The cells were subject to immunoblot analysis to investigate whether the change of MMP-2, MMP-9, and Snail is associated with that of p53 or PARP through knockdown of p53. β-actin was used as a normalization control. Note that the typical metastasis-related proteins, MMP-2, MMP-9, and Snail, are not associated with p53 or PARP.

after 24-h treatment with 8-MOP concentrations of 100, 200, and 300 μM was inhibited by 63.4%, 84.5%, and 99.9%, respectively.

8-MOP-induced apoptosis can be enhanced by a combination treatment with the chemotherapeutic drug of similar mechanism of action, gemcitabine

Since the combination chemotherapy of 5-FU and a platinum analog has been established as a first-line global standard regimen for treatment of metastatic gastric cancer, similar research is ongoing for clinical trials (Takashima *et al*., 2009). As the effects of 8-MOP were beneficial, especially for the metastasis of gastric cancer cells, the concept of combination therapy was assessed using gemcitabine, the first-line chemotherapy with an mechanism of action similar to that of 8-MOP (DNA damage) for the treatment of ovarian cancer (Lund *et al*., 1993). In this regard, we examined the potential of combination chemotherapy of SNU1 cells with 100 μM 8-MOP and 1 μM gemcitabine, neither of which caused complete cell death *in vitro*. As shown in Fig. 6, when the cells were simultaneously treated with 8-MOP and gemcitabine, activated caspase-3 was examined only with combination treatment. In contrast, MMP-2 suppression appeared to be affected by the presence of either 8-MOP or gemcitabine, without a combination effect. These results suggest that the combination treatment can enhance apoptosis without affecting cell mobility.

DISCUSSION

8-MOP is the parent compound that naturally occurs in traditional medicinal plants from the historical period. Due to the nature of naturally occurring phytochemicals, orally administered 8-MOP is safer for humans, even when used as a food supplement or chemotherapy, with fewer side effects compared to other chemotherapeutic chemicals. To date, 8-MOP has been studied as a component of the PUVA therapy. In contrast, diverse animals and human cancer cells were tested to study their sensitivity to 8-MOP alone without UVA. Recently, research areas have been extended to study other effects of 8-MOP or to find other sensitive tissues on the skin. For example, the antimetastatic effect of 8-MOP has been reported in hepatocellular carcinoma (Xiong *et al*., 2016). In addition, the anti-inflammatory and antioxidant effects of 8-MOP have been reported in osteoarthritis (Li *et al*., 2021). However, little is known about the effects of 8-MOP on human gastric cancer cells. In this study, we investigated, for the first time, the mechanisms underlying 8-MOP-induced cytotoxicity in the human gastric cancer cell line, SNU1. Specifically, treatment with 8-MOP induced expression of p53 and inhibited expressions of MMP-2, MMP-9, and Snail, leading to increased apoptosis and decreased cell mobility in the human gastric cancer cell line, SNU1. Moreover, our finding provides us the evidence that 8-MOP can be used as a combination treatment with available conventional first-line chemotherapy. In this regard, this study is valuable as it demonstrates the potential of 8-MOP for the treatment of human gastric cancer.

p53 and caspase-3 are well-known genetic guardians and apoptosis effectors. Moreover, downregulation of p53 has been well documented in gastric cancer (Fenoglio-Preiser *et al*., 2003). Thus, our finding of p53- and caspase-3 dependent signaling in the 8-MOP-induced G1-arrest and apoptosis effects appears to be normal. However, the association between p53 upregulation and caspase-3 activation and the effects of 8-MOP has been reported for the first time. Moreover, we confirmed this finding through loss-of-function analysis using p53 knockdown by siRNA as well as the apoptosis inhibitor Z-VAD-FMK. In addition, treatment with 8-MOP inhibited metastasis by downregulating MMP-2, MMP-9, and Snail in a dose-dependent manner. These results are in accordance with the results of a previous report stating that MMP-2, MMP-9, and Snail are associated with the metastasis of gastric cancer (Shin *et al*., 2012). Furthermore, the 8-MOP-induced G1-arrest and apoptosis are not associated with the 8-MOP-reduced metastatic ability, as determined by the finding that p53 upregulation is only responsible for the former effects, but not for the latter. This serendipitous finding endows 8-MOP with the potential of a combination treatment with a conventional first-line chemotherapeutic drug, gemcitabine, as 8-MOP can be used at the lowest concentration without losing its anti-metastatic effects.

In this study, we investigated, for the first time, the mechanisms underlying 8-MOP-induced cytotoxic and anti-metastatic effects on the human gastric cancer cell line, SNU1. Overall, we demonstrated the potential use of the phytochemical 8-MOP in the treatment of human gastric cancer.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

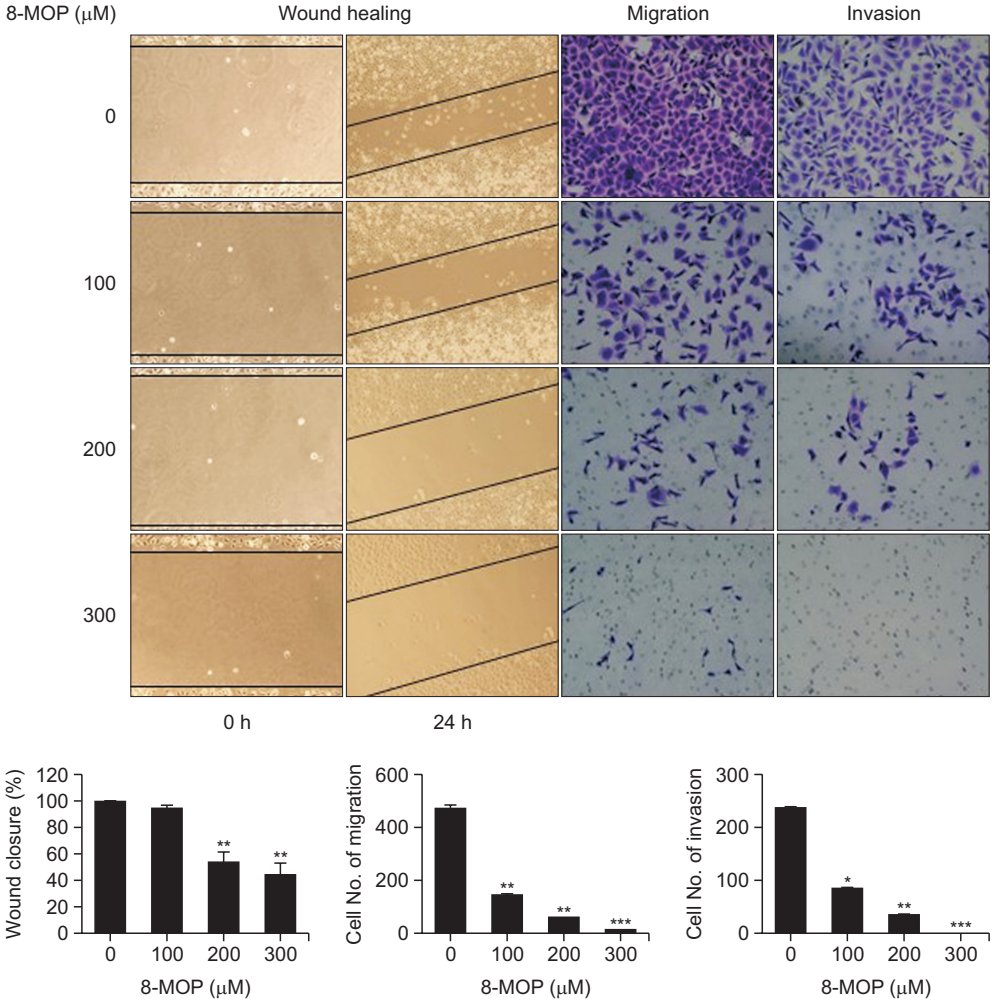

Fig. 5. 8-MOP-reduced wound healing, cell migration, and cell invasion. SNU1 cells were cultivated to confluence with 0, 100, 200, or 300 μM of 8-MOP treatment and subject to wound healing, cell invasion, and cell migration assays. The wound healing assay was performed at 0 and 24 h and the degree of recovery was compared with each other. For migration and invasion assays, cells were seeded in the absence and presence of Matrigel, respectively. The assay data was visualized into graph using GraphPad Prism 5.0, compared with controls. *n*=3; **p*<0.05, ***p*<0.01, or ****p*<0.001 *vs* untreated control.

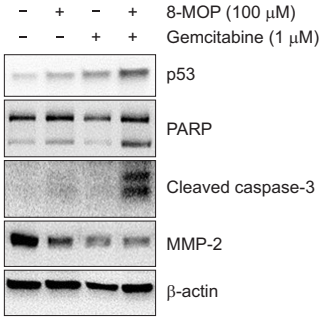

Fig. 6. Enhancement of the 8-MOP-induced apoptosis by a combination treatment with gemcitabine. SNU1 cells were treated with or without 8-MOP concentration of 100 μM in the presence or absence of 1 μM of gemcitabine for 24 h and subject to immunoblot analysis to detect the change in expression of p53, PARP, cleaved caspase-3, and MMP-2. β-actin was used as a normalization control. Note that the combination treatment with 8-MOP and gemcitabine enhanced the 8-MOP-induced apoptosis.

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