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Econazole Induces p53-Dependent Apoptosis and Decreases Metastasis Ability in Gastric Cancer Cells

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Abstract

Econazole, a potent broad-spectrum antifungal agent and a Ca^{2+} channel antagonist, induces cytotoxicity in leukemia cells and is used for the treatment of skin infections. However, little is known about its cytotoxic effects on solid tumor cells. Here, we investigated the molecular mechanism underlying econazole-induced toxicity *in vitro* and evaluated its regulatory effect on the metastasis of gastric cancer cells. Using the gastric cancer cell lines AGS and SNU1 expressing wild-type p53 we demonstrated that econazole could significantly reduce cell viability and colony-forming (tumorigenesis) ability. Econazole induced G0/G1 phase arrest, promoted apoptosis, and effectively blocked proliferation- and survival-related signal transduction pathways in gastric cancer cells. In addition, econazole inhibited the secretion of matrix metalloproteinase- 2 (MMP-2) and MMP-9, which degrade the extracellular matrix and basement membrane. Econazole also effectively inhibited the metastasis of gastric cancer cells, as confirmed from cell invasion and wound healing assays. The protein level of p53 was significantly elevated after econazole treatment of AGS and SNU1 cells. However, apoptosis was blocked in econazole-treated cells exposed to a p53-specific small-interfering RNA to eliminate p53 expression. These results provide evidence that econazole could be repurposed to induce gastric cancer cell death and inhibit cancer invasion.

Key Words: Apoptosis, Econazole, Gastric cancer, Invasion, Migration, p53

INTRODUCTION

Econazole (EN), an imidazole compound, is commonly used as an antifungal agent for the treatment of vulvar candidiasis (Osser *et al*., 1991) and superficial fungal infections (Aron-Brunetiere *et al*., 1977; Prajna *et al*., 2003). However, econazole and imidazole-related compounds exhibit other properties that may be beneficial for cancer therapy. For instance, econazole exerted potential anti-inflammatory effects against mouse aortic rings and cultured J774 cells, probably through the functional inactivation of nitric oxide (NO) synthase (Bogle and Vallance, 1996). In another study, econazole-treated NS1 myeloma cell lines showed reduced proliferation, viability, survival, and dose-dependent changes in DNA synthesis (Denyer *et al*., 1985). Imidazole antifungal drugs are known to inhibit the proliferation and invasion of breast cancer cells (Bae *et al*., 2018) and suppress phosphoinositide 3-kinase (PI3K) activity and cause apoptosis of lung cancer cells (Dong *et al*., 2017). In PC3 human prostate cancer cells, econazole increased the intercellular concentration of Ca^{2+} by stimulating Ca^{2+} influx into cells and Ca^{2+} release from the endoplasmic reticulum via a phospholipase C (PLC)-independent mechanism and inhibited cell proliferation in a dose-dependent manner (Huang *et al*., 2005). Anti-fungal azole compounds such as imidazole have been reported to inhibit the conversion of lanosterol to ergosterol by interacting with lanosterol 14-α demethylase (a CYP450 enzyme), thereby lowering the synthesis of cell membranes and causing seepage of cellular components (Jackson *et al*., 2000; Strushkevich *et al*., 2010; Warrilow *et al*., 2010).

Several clinical studies have supported the potential anti-tumor effects of antifungal agents. For instance, the combination of microtubule-active drugs, vinblastine, and paclitaxel with ketoconazole was reported to exert beneficial effects against hormone-independent metastasis of prostate cancer

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(Blagosklonny *et al*., 2000). In clinical studies, ketoconazole greatly reduced the need for painkillers in patients with reduced levels of serum prostatic acid phosphatase and testosterone (Trachtenberg and Pont, 1984). Ketoconazole inhibited the metastasis of hepatic cancer from human pancreatic adenocarcinoma (Tzanakakis *et al*., 1990), and ketoconazole treatment significantly reduced pulmonary metastasis in a nude mouse melanoma model (Nardone *et al*., 1988).

In this study, gastric cancer was chosen as a model to test the efficacy of econazole and to understand its underlying mechanism of action. Gastric cancer is the fifth most frequent type of cancer and the third most common cause of cancer-related deaths worldwide. Of the 1,000,000 people diagnosed with stomach cancer each year, 783,000 die due to related causes (Bray *et al*., 2018). The incidence rate is particularly high in Asian countries, including Korea. In 2018, gastric cancer was the second most common newly diagnosed cancer among Korean males (15.2%) and the third most common cancer among Korean females (9.5%) (Jung *et al*., 2018).

In colon cancer, econazole is thought to exert its effects by interacting with the protein p53 (Ho *et al*., 2005). p53 is a major tumor suppressor and modulates several important biological processes, including apoptosis, cell cycle, angiogenesis, and genetic stability (Vogelstein *et al*., 2000). The expression of p53 is suppressed in the intestinal metaplasia premalignant lesions of gastric cancer (Busuttil *et al*., 2014). About 50% of patients with gastric cancer exhibit genetic and epigenetic changes, which cause p53 inactivation (Imazeki *et al*., 1992; Gabbert *et al*., 1995; Bellini *et al*., 2012). Activation of the inactivated p53 protein to induce cancer cell death is a safer strategy than chemotherapy and has no cytotoxicity. This is a new application for natural products or conventional medicines that activate endogenous p53 expression.

Targeting the malignant invasion of cancer cells is important to combat cancer. The family of matrix metalloproteinases (MMPs) comprises 23 zinc-dependent endopeptidases that are involved in the degradation of the extracellular matrix (ECM) and are important for cancer progression, cell migration, invasion, and adhesion, and angiogenesis (Gialeli *et al*., 2011). Given their the unique ability to break down the major components of the basement membrane, the 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). Therefore, evaluation of the ability of econazole to inhibit MMP-2 and MMP-9 is desirable. Wound healing and invasion assays as well as zymography were performed to confirm the metastatic capacity of gastric cancer cells.

Here, we confirmed the inhibitory effect of econazole on the proliferation of various cancer cell lines, including gastric cancer, hepatic cancer, lung cancer, and breast cancer. Econazole also inhibited the secretion of MMP-2 and MMP-9, which are associated with cancer metastasis. Based on recent reports, we investigated whether econazole activates p53 in gastric cancer cell lines and observed that econazole mediated p53 activation in a dose-dependent manner and induced apoptosis of gastric cancer cells. Suppression of p53 levels with a p53-specific small-interfering RNA (siRNA) prevented the econazole-mediated death of cancer cells.

MATERIALS AND METHODS

Cell culture and reagents

The human gastric cancer cell lines AGS (wild-type p53) and SNU1 (wild-type p53) were purchased from the Korean Cell Line Bank (Seoul, Korea) and cultured in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% fetal bovine serum (FBS; Gibco, MD, USA) and 1% antibiotic-antimycotic solution. Cells were incubated at 37°C with 5% CO_2. Econazole, fluconazole, miconazole, and Z-VAD-FMK were purchased from Selleckchem (TX, USA). The reagents were diluted in dimethyl sulfoxide (DMSO) to obtain a final concentration of 100 mM.

Proliferation assay

The cytotoxicity of econazole against AGS and SNU1 cells was determined using the Cell Counting Kit-8 (CCK-8) assay (Sigma-Aldrich, St. Louis, MO, USA), performed according to the manufacturer's instructions. Cells were seeded in 96-well plates and incubated for 18 h, followed by treatment with fluconazole, miconazole, and econazole at different doses up to 100 μM for 24, 48, and 72 h. Following treatment, the cells were incubated with 20 μL of CCK-8 solution for 1-2 h, and the absorbance was measured at 460 nm wavelength using a microplate reader (SpextraMaxi3x, Molecular Devices, CA, USA).

Colony formation assay

AGS cells (500 cells) were seeded in six-well plates and cultured for 14 days. The cells were fixed with 4% paraformaldehyde and stained with a freshly prepared 0.01% crystal violet solution for 10 min. Following rinsing with distilled water, the colonies that formed in each well were counted using an inverted microscope (DMi1, Leica, Mannheim, Germany) under 40× magnification. Each experiment was performed in triplicate.

Flow cytometry

Cells (1×10^6) were treated with econazole for 24 h and then trypsinized. The cells were fixed and permeabilized with 70% methanol at –20°C overnight. After washing with ice-cold phosphate-buffered saline, the cells were incubated with a propidium iodide (PI) solution (0.2 mg/mL; RNase: 20 μg/mL and 0.1% Triton X-100) for 30 min at room temperature in the dark. Flow cytometry was performed using a FACSCalibur (BD, CA, USA). Ten thousand events per sample were counted in duplicate experiments.

Western blot analysis

Equal amounts of protein (measured with a Bradford assay) were loaded on 10-15% sodium dodecyl sulfate polyacrylamide gels, and the separated bands were transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% non-fat milk in Tris-buffered saline and Tween-20 buffer and incubated with antibodies against cleaved caspase-3, poly (ADP-ribose) polymerase (PARP) (Cell Signaling, MA, USA), β-actin, and p53 (Santa Cruz Biotechnology, CA, USA) at 4°C overnight. After washing, the blots were incubated with horseradish peroxidase-labeled secondary antibodies at room temperature for 2 h and signals were visualized using enhanced chemiluminescence LuminoGraph I (ATTO, Tokyo, Japan).

RNA interference

Cells were transiently transfected with siRNA using Lipofectamine RNAi MAX reagent (Invitrogen, Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol. The siRNA sequence for the transfection was: p53-siRNA, 5′-ACUCCACACGCAAAUUUCCTT-3′.

Invasion assay

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. AGS cells were seeded in the insert (1×10^3 cells/each insert) and treated with 1, 5, or 10 μM of econazole in RPMI-1640 medium without FBS. 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 of five random visual fields under a microscope were counted and averaged. All experiments were carried out in triplicate and repeated thrice.

Wound healing assay

AGS cells were seeded into six-well plates to obtain a confluent monolayer. After overnight incubation, a straight line was scratched in the middle of the cell monolayer with a pipette tip. The cells were washed with 1 mL PBS to remove cellular debris, placed in 2 mL of medium, and treated with 1, 5, or 10 μM econazole. After 24 h incubation, the width of the scratched wound was measured and compared to that at 0 h.

Zymography

Approximately 80% confluent AGS and SNU1 cells were

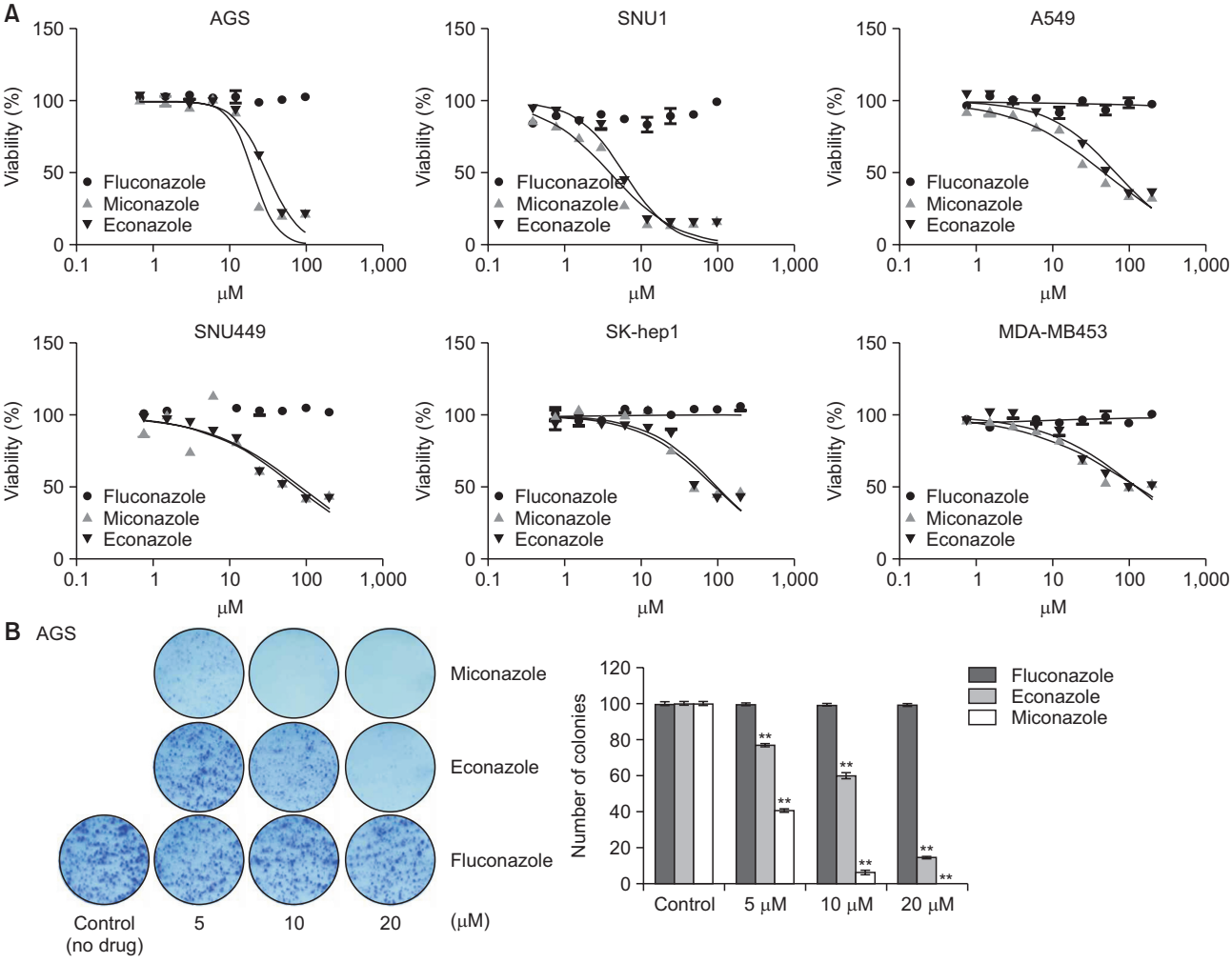

Fig. 1. Econazole induces the apoptosis of gastric cancer cell lines. (A) AGS, SNU1, A549, SK-hep1, SNU449, and MDA-MB-453 cells were cultured in the presence of increasing concentrations of econazole. Cell viability was determined with a CCK-8 assay after 48 h. (B) AGS cells were pre-treated with the indicated concentrations of fluconazole, econazole, and miconazole for 24 h. After incubation for 15 days, control (No drug) group colonies with more than 100 colonies were counted. Econazole induced apoptosis in AGS and SNU1 gastric cancer cells. (C) Cell cycle analysis: AGS and SNU1 cells were treated with econazole at different doses for 24 h and were subjected to flow cytometry analysis. (D) Apoptosis analysis by Annexin V-PI staining. (E) Apoptosis analysis by western blotting. Data are presented as the mean ± standard deviation (n=3), *p<0.05, **p<0.005.

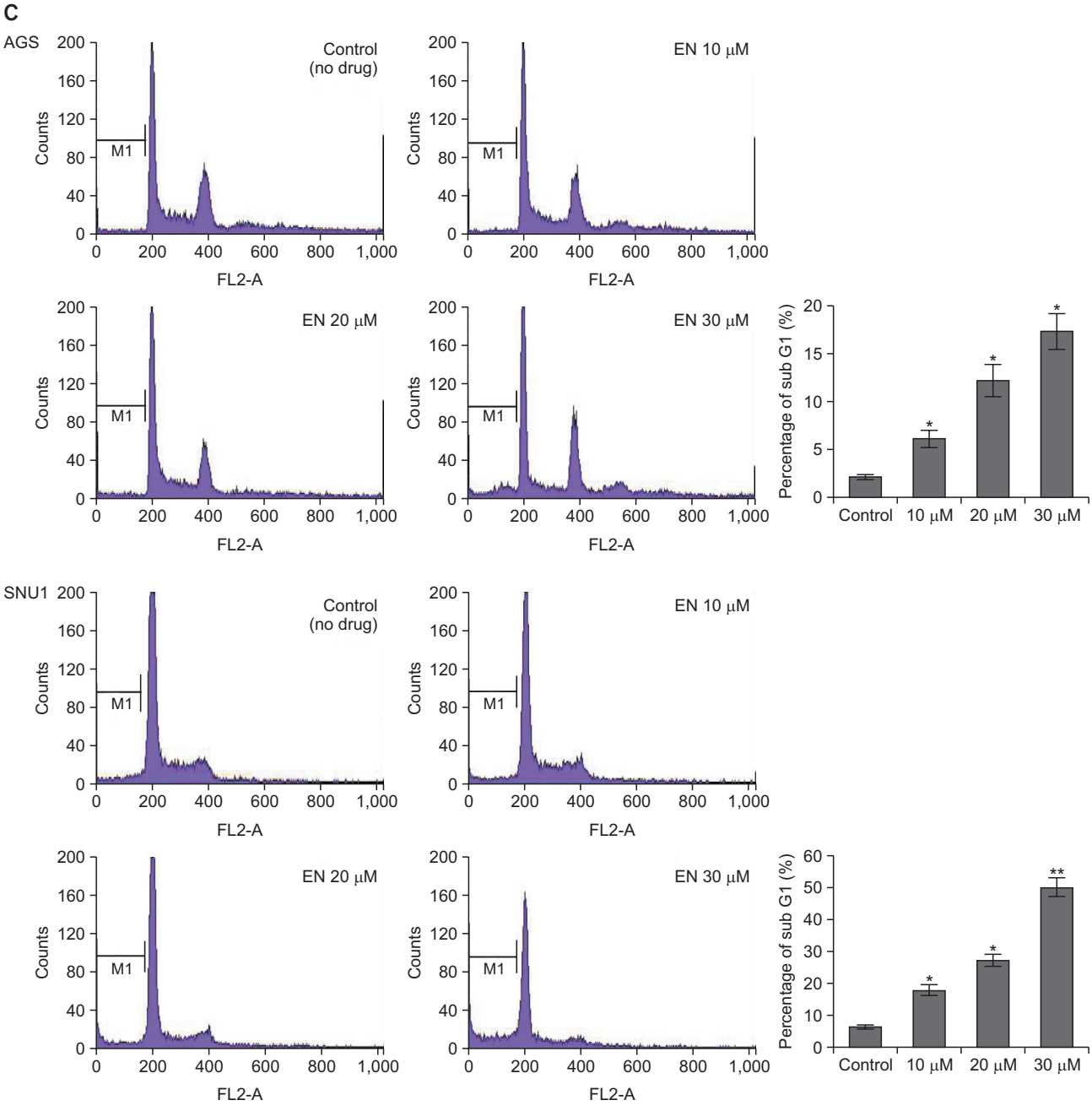

Fig. 1. Continued.

seeded in 60-mm dishes and incubated overnight at 37°C. The cells were washed with PBS and incubated in serum-free medium with 5, 10, or 20 μM econazole at 37°C for 24 h. To analyze the expression of MMP-2 and MMP-9, the medium was harvested and the protein concentration was measured with the Bradford assay. Protein (40 μg) was loaded on 10% SDS-PAGE gels containing 0.1% gelatin. The gel was washed twice with a renaturing buffer (2.5% Triton X-100) and distilled water and incubated in a developing buffer (50 mM Tris-Cl pH 7.6, 5 mM $CaCl_2$•$2H_2O$) at 37°C for 24 h. The gel was stained with 0.5% Coomassie blue for 1 h and washed with a de-staining buffer. The bands were visualized using LuminoGraph I (ATTO).

Statistical analyses

Statistical comparisons were performed using unpaired, two-tailed Student's *t*-test. Values of $p<0.05$ were considered statistically significant.

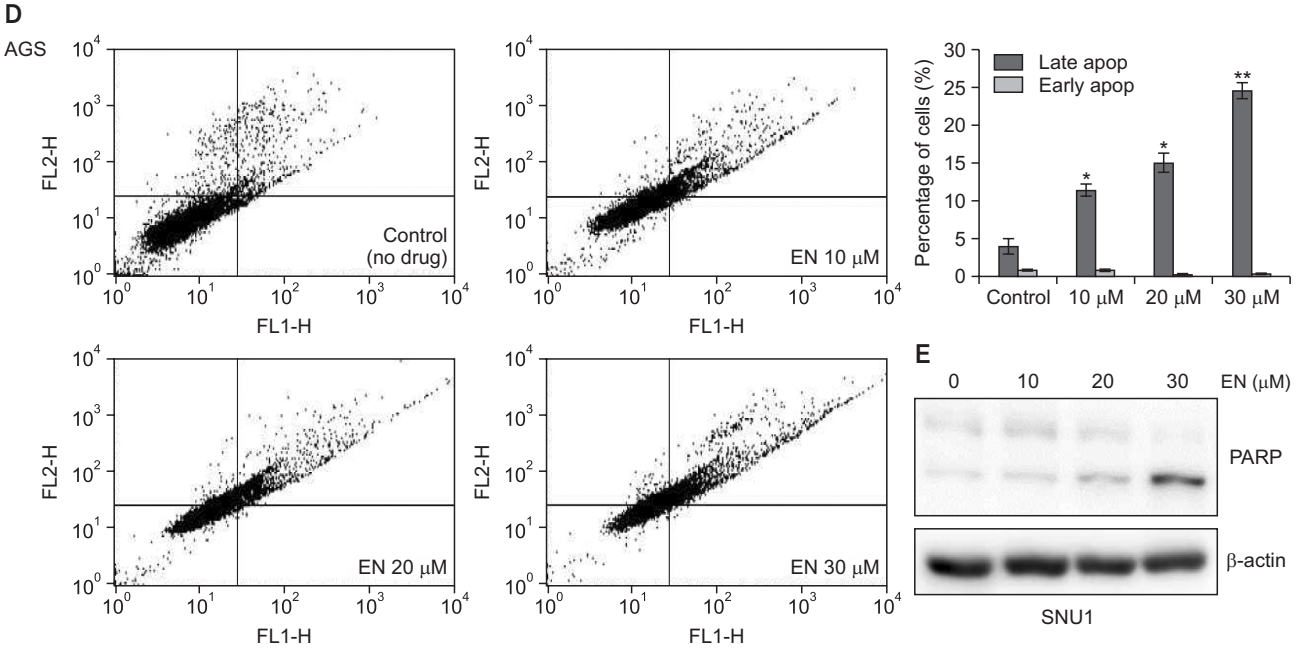

Fig. 1. Continued.

RESULTS

Econazole inhibits the viability of several cancer cell lines and induces apoptosis of gastric cancer cells

We tested the anticancer effects of three compounds using a CCK-8 assay. Two of the three compounds decreased the viability of all six cancer cell lines tested. The viabilities of the AGS and SNU1 cell lines were dramatically reduced after treatment with econazole and miconazole. Among the three compounds, econazole and miconazole showed the strongest dose-dependent anticancer effects (econazole IC50: 19.1 μM in AGS, 5 μM in SNU1, 69 μM in A549, 75.2 μM in SNU449, 93.5 μM in SK-hep1, and 130 μM in MDA-MB-453; miconazole IC50: 9.0 μM in AGS, 4.1 μM in SNU1, 49.3 μM in A549, 86.4 μM in SNU449, 100.4 μM in SK-hep1, and 126.5 μM in MDA-MB-452) (Fig. 1A). Fluconazole had no effect on the viability of these six cancer cell lines. A colony formation assay revealed a significant decrease in the number of AGS cells after treatment with econazole and miconazole (Fig. 1B). These results indicate that econazole and miconazole dramatically inhibited the tumorigenesis of gastric cancer cells and may potentially serve as powerful anticancer drugs. Miconazole had a better inhibitory effect on cell viability but is known to cause adverse side-effects such as dizziness, hypoesthesia, and decrease white blood cell count (Yan *et al*., 2016). Therefore, miconazole was not tested in detail in this paper. To investigate the mechanism underlying the inhibition of cancer cell viability by econazole, we performed Annexin V/PI flow cytometry assays. A significant dose-dependent increase in the number of AGS and SNU1 cells at the sub-G1 phase were observed following econazole treatments (Fig. 1C). Apoptotic death and PARP cleavage in response to econazole treatment were assessed in AGS and SNU1 cells using Annexin V/PI staining (Fig. 1D) and western blot analysis (Fig. 1E). The cells treated with econazole showed positive staining for Annexin V and PI. We also found that exposure of gastric cancer cell lines to econazole resulted in a dose-dependent increase in the cleavage of PARP compared to untreated cells. These results indicate that econazole induced death via apoptosis of gastric cancer cells.

Econazole solely promotes apoptosis in gastric cancer cells

To determine whether econazole treatment induces caspase-dependent apoptosis, we treated AGS and SNU1 cells with econazole for 24 h and performed western blotting to detect the expression of cleaved PARP and caspase-3. The levels of cleaved PARP and caspase-3 were higher in SNU1 cells treated with 20 μM econazole for 24 h (Fig. 2A). AGS cells were pretreated with the pan-caspase inhibitor Z-VAD-FMK and then exposed to econazole after 1 h to confirm caspase-dependent apoptosis. As a result, we found that pretreatment with 50 μM Z-VAD-FMK decreased the activation of procaspase-3 and PARP in econazole-treated cells. Previous studies have shown that econazole induces apoptosis in NSCLC through the PI3K and protein kinase B (AKT) signaling pathways. Hence, we investigated the changes in the expression of AKT and PI3K (Dong *et al*., 2017) and found that the expression levels of phosphorylated AKT (pAKT) and pPI3K were completely downregulated by econazole (Fig. 2B). In addition, caspase-3 and PARP cleavage mediated by econazole was completely abrogated by the pan-caspase inhibitor Z-VAD-FMK. Econazole treatment increased cell cycle arrest in the sub-G1 phase (30%), while pre-treatment with Z-VAD-FMK prevented the econazole-mediated accumulation of AGS cells in the sub-G1 phase (Fig. 2C). These results indicate that econazole induced cell death through caspase-dependent apoptosis. To reaffirm that the death was caused by apoptosis, cells were treated with autophagy and necrosis inhibitors (3-MA and necrostatin). Only Z-VAD-FMK was found

to inhibit PARP cleavage (Fig. 2D). Thus, econazole induced caspase-dependent apoptosis of gastric cancer cell lines.

Econazole decreases the migration and invasion of AGS cells

Next, we examined the effect of econazole on the motility of cancer cells via cell invasion, Zymography, and wound healing assays. Cell migration was 60.5% in the group treated with 1 μM econazole and decreased to 15.5% after treatment with 5 μM econazole; no cell migration was observed following treatment with 10 μM econazole (Fig. 3A). The wound healing rate in AGS cells was significantly reduced after treatment with econazole in a dose-dependent manner. After 24 h, the wound healing rate was 73.5%, 61.8%, and 20.6% following treatment with 1, 5, and 10 μM econazole, respectively (Fig. 3B). Based on these results, we suspect that the function of econazole may be related to MMPs. MMP-2 and MMP-9 are associated with the degradation of ECM and play a critical role in the invasion and metastasis of tumors. Therefore, we examined whether econazole could inhibit the secretion of MMP-2 and MMP-9 in AGS and SNU1 cells. The inhibition of MMP-2 and MMP-9 secretion was confirmed by Zymography (Fig. 3C). In particular, the secretion of MMP-2 and MMP-9 was completely inhibited in SNU1 cells treated with 20 μM econazole.

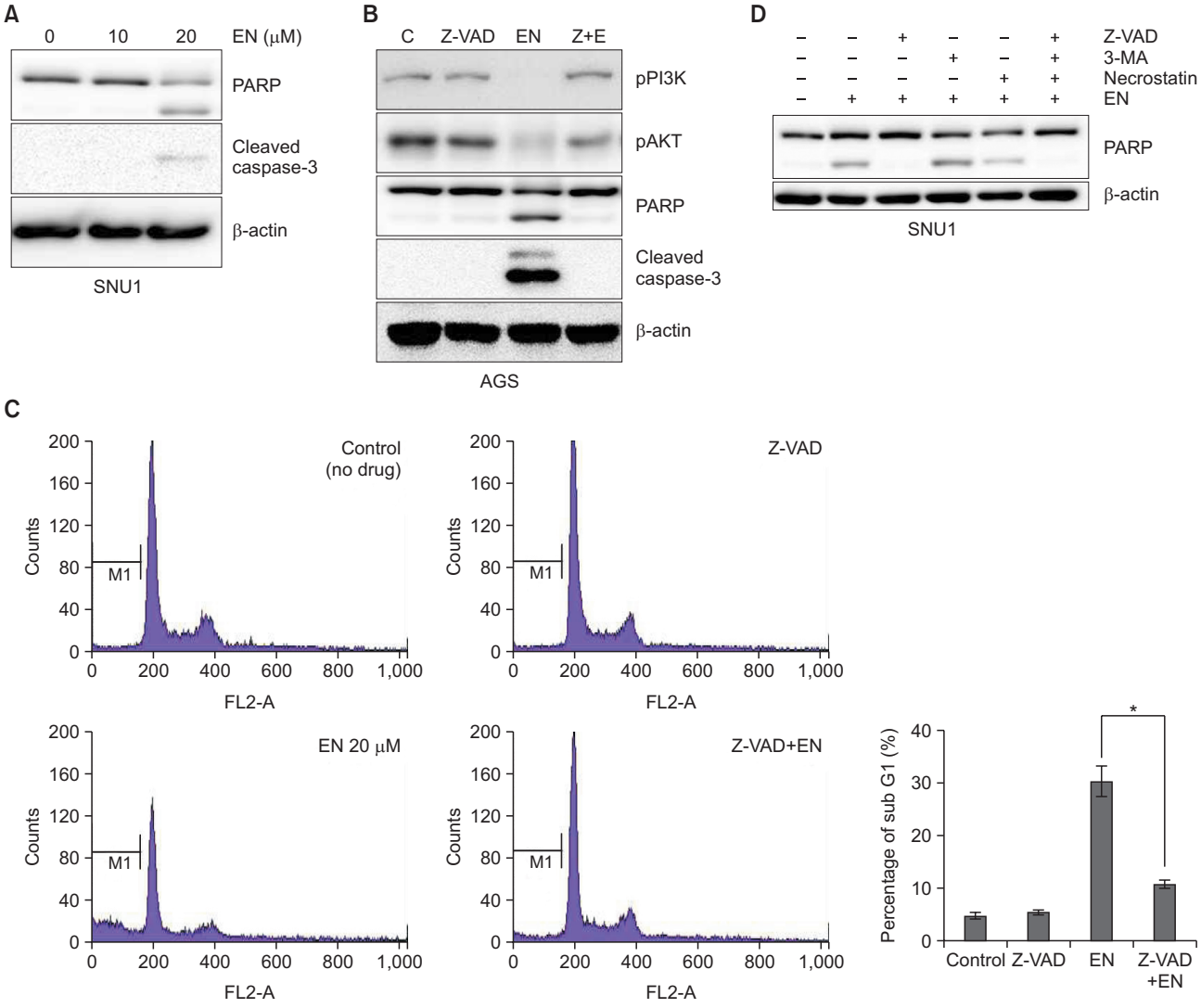

Fig. 2. The effect of 3-MA, Z-VAD-FMK, and necrostatin on econazole-induced apoptosis. (A) SNU1 cells were treated with increasing concentrations of econazole for 24 h. The cells were harvested, and the cell lysates were subjected to immunoblot analysis for the expression of cleaved PARP and caspase-3. β-actin was used as a loading control. (B) SNU1 cells were treated with a pan-caspase inhibitor, Z-VAD-FMK, for 2 h before incubation with 20 μM econazole for 24 h. The cells were lysed and subjected to immunoblot analysis for the expression of pAKT, pPI3K, cleaved caspase-3, and PARP and (C) cell cycle distribution using FACS. (D) Percentage of cells in sub-G1 phase after treatment with DMSO, econazole, or Z-VAD-FMK plus econazole treatment. Cells were incubated with a pan-caspase inhibitor (Z-VAD-FMK), PI3K inhibitor (3-MA), and necrosis inhibitor (necrostatin) for 2 h before treatment with 20 μM econazole for 24 h. The cells were lysed and subjected to immunoblot analysis for cleaved PARP expression. All figures show data from three independent experiments. Data are presented as the mean ± standard deviation (n=3), *p<0.05.

Econazole induces apoptosis through p53 and inhibits survival-related signaling pathways

Previous experiments confirmed that econazole reduced the expression of pPI3K and pAKT and promoted apoptosis. To confirm the effects of econazole on the signaling pathways related to cell growth and survival, western blot analysis was performed. As a result, the expression of pPI3K, pAKT, p-extracellular signal-related kinase (ERK), and p-signal transducer and activator of transcription 3 (STAT3) was reduced in an econazole dose-dependent manner (Fig. 4A). SNU1 cells, in particular, showed a significant decrease in the levels of pPI3K, pAKT, pERK, and pSTAT3 even at low doses of econazole. These effects may be related to the toxicity of econazole in SNU1 cells at low doses. SNU1 are floating cells unlike AGS, and may be more sensitive to the inhibitory effects of low-dose econazole. As econazole was shown to restore the expression of p53 in colon cancer, we investigated the effects of econazole on p53 expression in gastric cancer cells. The p53 protein levels in SNU1 and AGS cells treated with econazole increased in a dose-dependent manner (Fig. 4A). To confirm that SNU1 and AGS cell death induced by econazole correlated with p53 expression, we knocked down the endogenous expression of p53 using siRNA in SNU1 and AGS cells. Cells were transfected with scrambled-siRNA or p53-siRNA and then treated with econazole. Transfection with p53-siRNA resulted in decreased cell death and PARP and caspase-3 cleavage compared to scrambled siRNA treatment (Fig. 4B). Cell death was confirmed using flow cytometric analysis. There were fewer cells in the sub-G1 phase following econazole treatment in the p53-siRNA treatment group than in the scrambled siRNA treatment group (Fig. 4C). These results indicate that the induction of p53 expression by econazole may lead to the apoptosis of SNU1 and AGS cells.

DISCUSSION

The combination of ECF (epirubicin, cisplatin, and 5-FU), cisplatin, or oxaliplatin and 5-FU or capecitabine is commonly used with radiation as the first-line treatment for gastric cancer. These chemotherapeutic drugs exert side-effects, including nausea, vomiting, fatigue, loss of appetite, hair loss, diarrhea, mouth sores, and increased chance of infection, bleeding, or bruising after minor cuts or injuries (Takashima *et al*., 2009). p53, ras, raf, BRCA1, BRCA2, EGFR, PI3K, ALK, and MET that are frequently mutated in various carcinomas have been the common targets for the development of these drugs (Sanchez-Vega *et al*., 2018). Targeted therapeutic anticancer drugs that minimize side-effects and specifically kill cancer cells are constantly being developed; however, these

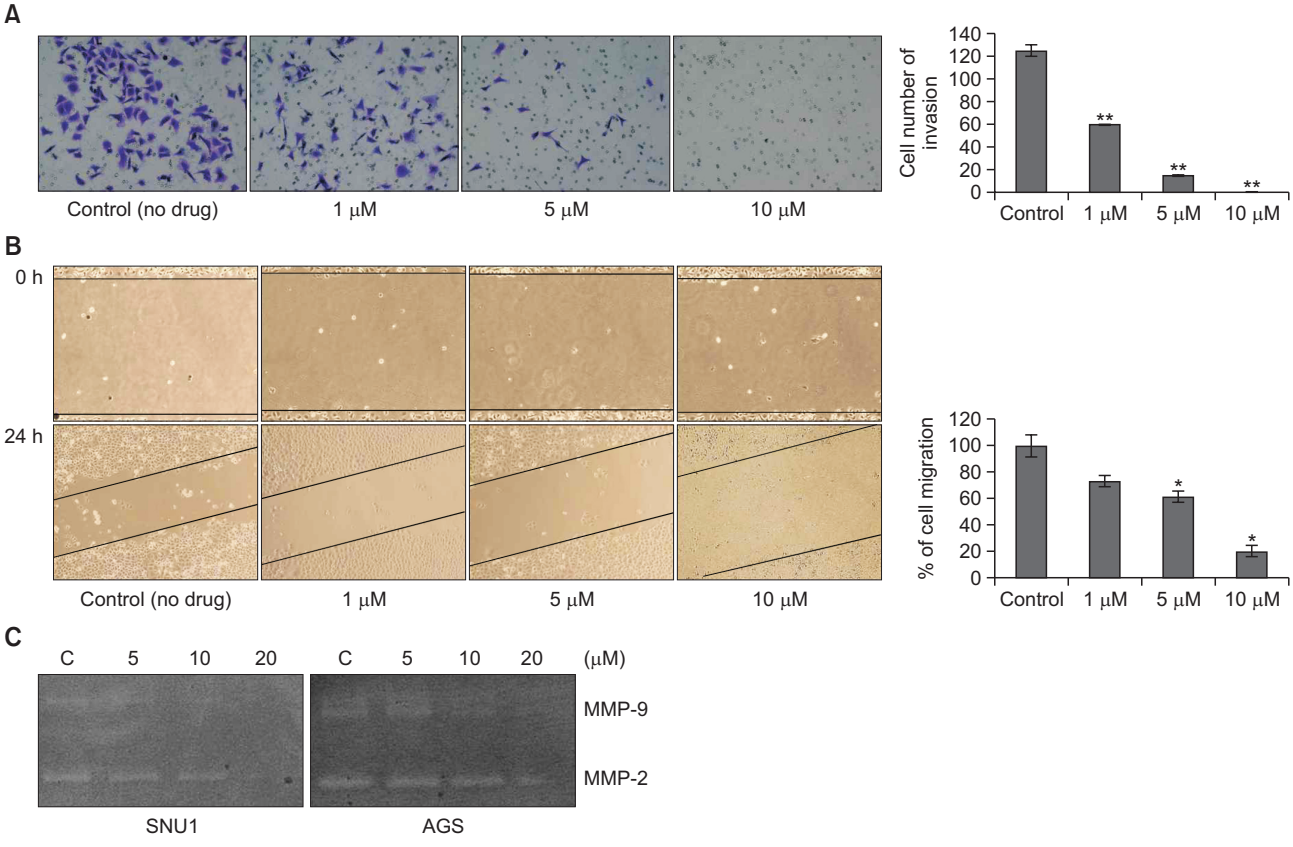

Fig. 3. Econazole inhibits gastric cancer cell migration and invasion. (A) AGS cells were seeded in 20% Matrigel-coated wells and cultured for 18 h, followed by incubation with different concentrations of econazole for 24 h. (B) Wound healing assay was performed with AGS cells at 0 and 24 h after econazole treatment. (C) Supernatants from AGS and SNU1 cells treated with different doses of econazole were spun down and their protein levels were quantified with the Bradford assay. The lysates containing 40 μg protein were loaded onto 10% SDS-PAGE gel containing 0.1% gelatin. Data are presented as the mean ± standard deviation (n=3), **p*<0.05, ***p*<0.005.

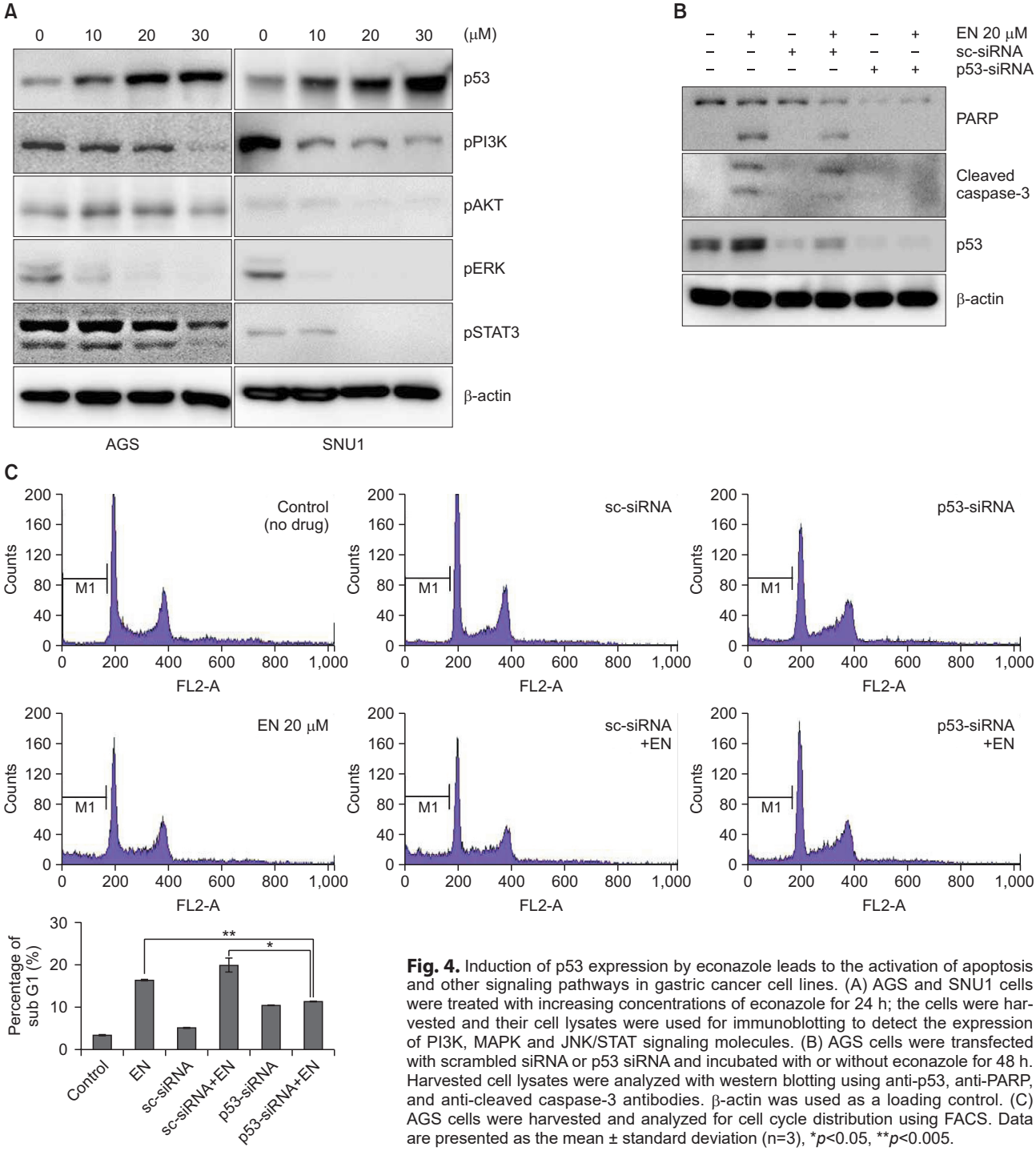

Fig. 4. Induction of p53 expression by econazole leads to the activation of apoptosis and other signaling pathways in gastric cancer cell lines. (A) AGS and SNU1 cells were treated with increasing concentrations of econazole for 24 h; the cells were harvested and their cell lysates were used for immunoblotting to detect the expression of PI3K, MAPK and JNK/STAT signaling molecules. (B) AGS cells were transfected with scrambled siRNA or p53 siRNA and incubated with or without econazole for 48 h. Harvested cell lysates were analyzed with western blotting using anti-p53, anti-PARP, and anti-cleaved caspase-3 antibodies. β-actin was used as a loading control. (C) AGS cells were harvested and analyzed for cell cycle distribution using FACS. Data are presented as the mean ± standard deviation (n=3), *p<0.05, **p<0.005.

drugs are expensive and necessitate several expensive tests to find the action points. Moreover, these targeted therapeutic anticancer drugs may not be effective against all cancer types and the underlying mechanisms of action of many drugs are still incompletely understood. In early gastric cancer, the expression of p53 is suppressed and the mutation frequency is low. However, the frequency of p53 mutation increases with the progression of cancer, and various mutations have already been reported (Gleeson *et al*., 1998; Tolbert *et al*., 1999; Shiao *et al*., 2000). This study was conducted to investigate the effects of econazole, a safe alternative to chemotherapy in cancer patients with wild-type p53, against cancer cells via p53 activation.

Econazole has been traditionally used for the treatment of superficial mycosis and pulmonary aspergillosis in middle-aged women (Hantschke *et al*., 1978). A recent study indi-

cated the cytotoxicity of econazole against cancer cell lines; however, the exact mechanism of action remains unclear. Econazole stimulated Ca^{2+} influx in the human prostate cancer PC3 cell line and induced cytotoxicity by releasing Ca^{2+} from the endoplasmic reticulum (Huang *et al*., 2005). Furthermore, it inhibited the proliferation of MCF-7 breast cancer cells *in vitro* and *in vivo* (Najid and Ratinaud, 1991; Cogswell *et al*., 2006; Sun *et al*., 2014; Bae *et al*., 2018) and induced apoptosis of non-small cell lung cancer (NSCLC) cells through the inhibition of the PI3Kα activity via AKT and B cell lymphoma 2 (Bcl2) (Dong *et al*., 2017). Econazole also induced the apoptosis of a colon cancer cell line by inducing G0/G1 cell cycle arrest (Ho *et al*., 2005). Despite these reports, all clinical trials with econazole have been limited to study its stability and efficacy against tinea pedis (called Athlete's foot) and in the form of combination therapy with benzydamine HCl 6mg vaginal pessary against vulvovaginal candidosis. We have been studying the repositioning of antifungal drugs for cancer treatment. As seen herein, antifungal drugs reduce viability and induce apoptosis of cancer cells. We found that econazole activated p53 and induced caspase-dependent apoptosis of gastric cancer cells. This seems safer than traditional chemotherapy, which causes unwanted cellular damage through inhibiting DNA replication or cell division.

Econazole inhibited signaling pathways related to cell proliferation and killed more than 80% of cancer cells at high concentrations (exceeding 30 μM), consistent with the observations reported in NSCLC, breast, prostate, and colon cells (Ho *et al*., 2005; Huang *et al*., 2005; Sun *et al*., 2014; Dong *et al*., 2017). In SNU1 cells, the expression of pPI3K, pAKT, and pERK was reduced by more than 50% after treatment with 10 μM econazole. A similar dose-dependent decrease in pPI3K, pAKT, pERK, and pSTAT3 levels was observed in AGS cells after econazole treatment along with an increase in the expression level of p53. In the experiment using siRNA to alter the expression level of p53, p53 was slightly increased after econazole treatment. This observation confirms the activation of p53 by econazole and that cancer cells are killed by the activated p53 protein. SNU1 and AGS cell lines are gastric cancer cells with no expression of wild-type p53. Malignant gastric cancer cells often carry mutanted p53, and we performed experiments to determine the effects of econazole on cancer cells with mutated p53. The treatment of p53-mutant N87 and SNU016 gastric cancer cell lines with econazole confirmed the absence of any change in their viability (Supplementary Fig. 1). Apoptosis was induced by econazole only in gastric cancer cells expressing wild-type p53. In addition, econazole inhibited the secretion of MMP-2 and MMP-9, which are important for cancer metastasis, and inhibited the overall ability for metastasis, as evident from the wound healing and invasion assays. Future studies are warranted to confirm whether this decline in metastatsis ability was mediated by the activation of p53.

In this study, we demonstrate the novel properties of econazole, which has fewer side-effects than the common chemotherapy drugs and is more stable in humans even when used as a supplement or enhancer of chemotherapy.

CONFLICT OF INTEREST

None.

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