

Metformin induces apoptosis and inhibits migration by activating the AMPK/p53 axis and suppressing PI3K/AKT signaling in human cervical cancer cells

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Received May 15, 2020; Accepted October 30, 2020

DOI: 10.3892/mmr.2020.11725

Abstract. Human cervical cancer is the fourth most common malignancy among women worldwide, and it is expected to result in 460,000 deaths per year by 2040. Moreover, patients with cervical cancer often display drug resistance and severe side effects; therefore, the development of effective novel chemotherapeutic agents is important. In the present study, the effects of metformin, a first-line therapeutic drug for type 2 diabetes mellitus, were evaluated in cervical cancer. Compared with the control group, metformin significantly inhibited cell viability and migration, and induced apoptosis and cell cycle arrest in human cervical cancer cell lines (CaSki and HeLa). Following metformin treatment, the protein expression levels of p-AMP-activated protein kinase (p-AMPK), which promotes cell death, and the tumor suppressor protein p-p53 were remarkably upregulated in CaSki and C33A cells compared with the control group. Furthermore, compared with the control group, metformin significantly suppressed the PI3K/AKT signaling pathway in CaSki, C33A and HeLa cells. Compound C (an AMPK inhibitor) significantly reversed the effects of metformin on CaSki, C33A and HeLa cell viability, and AMPK and p53 phosphorylation. The results of the present study suggested that metformin induced AMPK-mediated apoptosis, thus metformin may serve as a chemotherapeutic agent for human cervical cancer.

Introduction

Cervical cancer is the fourth most common malignancy in women worldwide, resulting in considerable economic and medical burden within society (1). Cervical cancer is a global public health issue (1) with the number of associated deaths per year estimated to reach 460,000 by 2040 (2). Cervical cancer treatment typically includes surgery, chemotherapy and radiotherapy; however, the treatment strategies are not always sufficient (3). Various chemical drugs, such as bevacizumab, topotecan hydrochloride and gemcitabine/cisplatin, are the first-line treatments used in cervical cancer chemotherapy; however, patients often display severe side effects and drug resistance, resulting in tumor recurrence and further progression (4,5). Therefore, improving the current understanding of the biological properties of cervical cancer cells and developing effective novel chemotherapeutic agents is important.

Metformin, a biguanide derivative approved by the US Food and Drug Administration, is a well-tolerated first-line therapy for type 2 diabetes mellitus (6). Clinical and laboratory studies have demonstrated that metformin inhibits cellular proliferation, and induces apoptosis and cell cycle arrest *in vitro* (7-13). Moreover, metformin reduced the growth of experimental tumors *in vivo*, including in prostate, lung, breast, colon and pancreatic cancer, as well as in oral squamous cell carcinoma and melanoma (7-13). Moreover, several studies have reported that in cancer therapeutics, metformin exerts its effects via several molecular signaling pathways, including the AMP-activated protein kinase (AMPK), mTOR, insulin-like growth factor-1, JNK/p38 MAPK, human epidermal growth factor receptor-2 and NF- κ B signaling pathways (14-20). However, Kowall *et al* (21) and Iliopoulos *et al* (22) revealed that when used alone, metformin is not an effective anticancer treatment for colorectal, lung, breast or prostate cancer; however, when combined with multiple chemotherapeutic agents, metformin can effectively reduce tumor progression in various types of cancer. *In vitro* and *in vivo* studies have indicated that

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Key words: metformin, apoptosis, migration, AMPK-activated protein kinase/p53, PI3K/AKT, cervical cancer

when combined with caffeic acid or nelfinavir, metformin synergistically inhibits cancer cell proliferation and tumor growth (23,24). The combined use of metformin and chemotherapeutics (including, gemcitabine, paclitaxel/carboplatin, megestrol acetate and erlotinib) is being investigated in several ongoing phase I/II clinical trials (clinicaltrials.gov) to assess whether these combinations increase patient survival and inhibit pancreatic (NCT02005419), ovarian (NCT02312261), endometrial (NCT01968317) and breast cancer metastasis (NCT01650506).

Pollak (25) revealed that the antineoplastic mechanisms underlying metformin may display indirect and/or direct effects on cancer biology. Indirectly, metformin binds components of respiratory complex I to limit mitochondrial oxidative phosphorylation, resulting in induced hepatic energy stress and reduced liver gluconeogenesis, which ultimately leads to a decrease in circulating glucose and insulin levels, and in turn may inhibit tumor growth in patients with insulin-responsive cancer (26). Regarding its direct effects on cancer, metformin may adequately accumulate in neoplastic tissues resulting in reduced ATP production, which triggers AMPK activation, but inhibits mTOR and fatty acid synthases expression, ultimately reducing cellular energy consumption and promoting energetic stress, resulting in tumor cell death (27,28). Another study revealed that metformin reduces ATP production, which when coupled with the loss of AMPK, p53 or liver kinase B1 (LKB1) function, energy deficient tumor cells may not exhibit a high compensatory rate of glycolysis, resulting in a lethal energetic crisis and cytotoxic effects (29-31). Thus, understanding the mechanisms underlying the therapeutic effects of metformin is crucial for the development of potential cervical cancer therapies.

AKT is a serine/threonine kinase with a key role in the PI3K/AKT signaling pathway, which mediates various biological functions, including cell survival, proliferation, apoptosis, angiogenesis, glucose metabolism, mitochondrial membrane gradient and protein synthesis (32,33). Increased AKT activity has been reported in 30-50% of breast, ovarian, prostate and pancreatic cancer tumors (34,35). The results of clinical studies indicated that the levels of phosphorylated (p)-AKT were also high in esophageal squamous cell carcinoma (90.4%), lung cancer (76.4%) and breast cancer (20-26%), which often resulted in drug resistance and lower patient survival rates (36-40). In the present study, the anti-cancer mechanisms underlying metformin were evaluated by performing an apoptosis assay, and the activation of the AMPK/p53 and PI3K/AKT signaling pathways in human cervical cancer cell lines (CaSki, C33A and HeLa) following metformin treatment were evaluated.

Materials and methods

Cell line and culture. The CaSki, HeLa and C33A human cervical cancer cell lines were purchased from the Bioresource Collection and Research Center. CaSki cells were cultured in RPMI-1640 (BioConcept AG) and HeLa and C33A cells were cultured in Eagle's minimum essential medium (MEM; HyClone: Cytiva) supplemented with 10% fetal bovine serum (FBS; HyClone: Cytiva) in a humidified incubator at 37°C with 5% CO₂, respectively.

Cell viability assay. Cells were seeded into a 96-well plate (2x10⁴ cells/well) in RPMI-1640 or MEM. Subsequently, cells were pre-treated with or without Compound C (an AMPK inhibitor; Merck KGaA) for 2 h at 37°C. CaSki and C33A were cells treated with 1 μM Compound C and HeLa cells were treated with 5 μM Compound C, followed by treatment with metformin (0-20 mM; Cayman Chemical Company) for 48 h at 37°C. Following treatment, 10 μl Cell Counting Kit-8 (CCK-8) solution (Dojindo Molecular Technologies, Inc.) was added to each well and incubated at 37°C for 1 h. Absorbance was measured at a wavelength of 450 nm using a FLUOstar Galaxy microplate reader (BMG Labtech GmbH).

Migration assay. Cell migration was assessed by performing a Transwell migration assay using Transwell chambers (24-well inserts; 8.0-micron PET; BD Biosciences). Cells were seeded (2x10⁴ cells/well) into the upper chamber in serum-free RPMI-1640 or MEM medium containing 0, 5 or 10 mM metformin; these concentrations of metformin having been selected based on the cell viability assay test. The lower chamber was filled with 700 μl RPMI-1640 or MEM medium supplemented with 10% FBS. Following incubation for 48 h at 37°C, a wet cotton swab was used to remove the non-migratory cells from the upper surface of the Transwell membrane. Subsequently, migratory cells were fixed with 3.7% formalin for 2 min and 100% methanol for 20 min at room temperature (RT). Migratory cells were stained with 0.1% crystal violet for 20 min at RT and observed using a BX61 fluorescence microscope (magnification, x100; Olympus Corporation).

Apoptosis and cell cycle analyses. Apoptosis and cell cycle analyses were performed using an FITC Annexin V Apoptosis Detection kit (BD Biosciences) and PI/RNase staining buffer (BD Pharmingen; BD Biosciences), respectively, according to manufacturer's instructions. Cells were seeded (1x10⁶ cells/well) into 6-well plates and treated with 0, 5 or 10 mM metformin for 48 h at 37°C. Subsequently, cells were collected, fixed with cold 70% ethanol at room temperature and stored at -20°C until analysis. Prior to analysis, cells were centrifuged at 400 x g for 10 min at 4°C and washed with cold PBS. For cell cycle analysis, cells were stained with 0.5 ml PI/RNase staining buffer for 15 min at RT in the dark. For apoptosis analysis, cells were double-stained with 5 μl Annexin V-FITC and 5 μl PI for 15 min at RT in the dark. Stained cells were analyzed using a Cytomics FC500 flow cytometer (Beckman Coulter, Inc.), CXP software (version 2.3; Beckman Coulter, Inc.) and late apoptosis was assessed.

Western blotting. Cells were seeded (2x10⁶ cells/dish) into 10-cm dishes, pre-treated with or without Compound C (1 or 5 μM) for 2 h at 37°C and incubated with 0, 5 or 10 mM metformin for 48 h at 37°C. Total protein was isolated from cells using RIPA buffer (EMD Millipore) and protein concentrations were determined using a BCA protein assay kit (Thermo Fisher Scientific, Inc.). Proteins (30 μg) were incubated at 95°C for 10 min, separated via 10-12% SDS-PAGE and transferred to 0.2-μm PVDF membranes (Bio-Rad Laboratories, Inc.). Following blocking with BlockPRO blocking buffer (Energen Biomedical Co., Ltd.) for 1 h at room temperature, the membranes were incubated

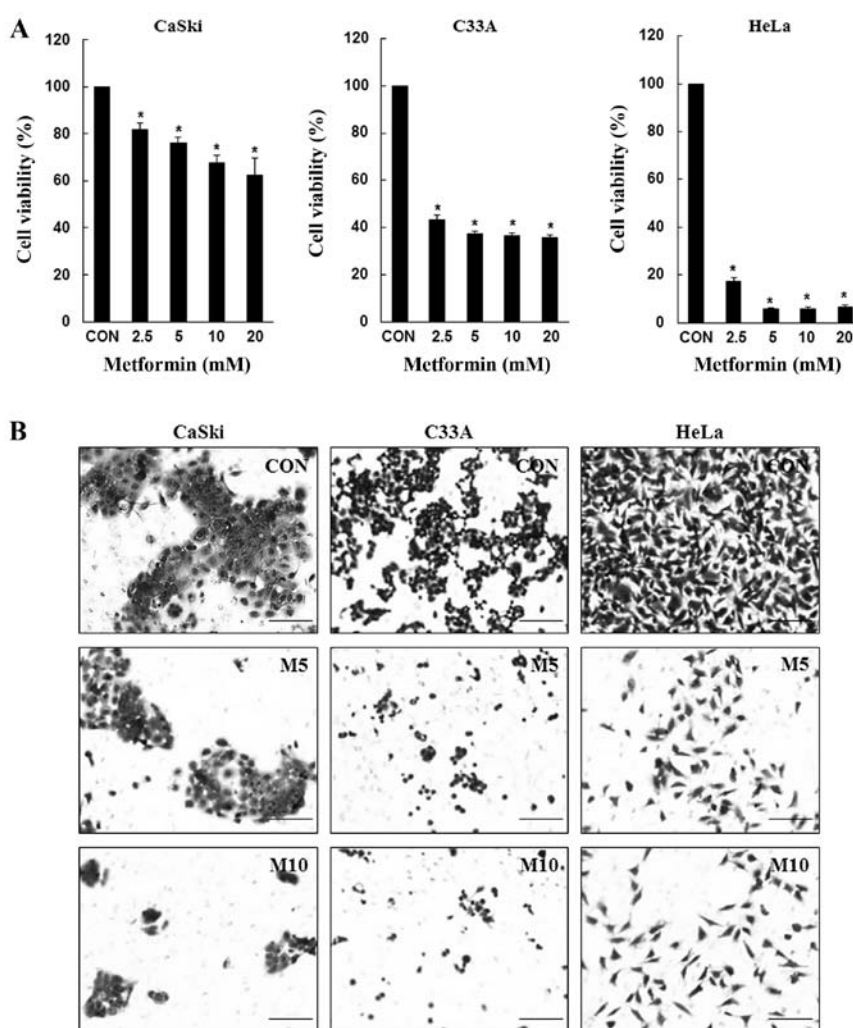


Figure 1. Metformin inhibits cervical cancer cell proliferation and migration. (A) CaSki, C33A and HeLa cells were treated with metformin (0-20 mM) for 48 h. Cell viability was determined by performing the Cell Counting Kit-8 assay. (B) CaSki, C33A and HeLa cells were treated with metformin (0-10 mM) for 48 h. Cell migration was assessed by performing a Transwell migration assay (scale bar, 200 μ m). Data are presented as the mean \pm SD from three independent experiments. * P <0.05 vs. CON. CON, 0 mM metformin; M5, 5 mM metformin; M10, 10 mM metformin.

overnight at 4°C with the following primary antibodies (all 1:1,000): Monoclonal anti-p-AMPK α (Thr172; cat. no. 2535; Cell Signaling Technology, Inc.), monoclonal anti-AMPK α (cat. no. 5832; Cell Signaling Technology, Inc.), polyclonal anti-p-p53 (cat. no. 9284; Cell Signaling Technology, Inc.), polyclonal anti-p53 (cat. no. 9282; Cell Signaling Technology, Inc.), monoclonal anti-Bcl-2 (cat. no. 15071; Cell Signaling Technology, Inc.), monoclonal anti-Bax (cat. no. 5023; Cell Signaling Technology, Inc.), monoclonal anti-cleaved caspase-3 (cat. no. 9664; Cell Signaling Technology, Inc.), monoclonal anti-p-AKT (Ser473; cat. no. 4060; Cell Signaling Technology, Inc.), monoclonal anti-Akt (cat. no. 4298; Cell Signaling Technology, Inc.), monoclonal anti-p-p70S6 kinase (p70S6K; Thr389; cat. no. 9234; Cell Signaling Technology, Inc.), monoclonal anti-p70S6K (cat. no. 2708; Cell Signaling Technology, Inc.), polyclonal anti-Bcl-2 antagonist/killer 1 (Bak; cat. no. GTX100063; GeneTex, Inc.), polyclonal anti-phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (PIK3CA; cat. no. NBP2-19804; Novus Biologicals, LLC) and monoclonal anti-GAPDH (cat. no. AC002; ABclonal Biotech Co., Ltd.). Following washing with TBST (Tris-buffered saline, 0.1% Tween-20), the membranes were

incubated with an IgG HRP-conjugated secondary antibody (polyclonal anti-mouse; cat. no. 115-035-003; 1:50,000; Jackson ImmunoResearch Laboratories, Inc.; polyclonal anti-rabbit; cat. no. 31460; 1:100,000; Thermo Fisher Scientific Inc.) for 1 h at room temperature. Proteins bands were visualized using enhanced chemiluminescence reagent (EMD Millipore). Densitometry was performed using Fusion-Capt Advanced FX7 software (version 16.08a; Labtech International, Ltd.) with GAPDH as the loading control.

Statistical analysis. All experiments were performed at least three times. Data are presented as the mean \pm standard deviation. Comparisons among groups were analyzed using one-way ANOVA followed by Bonferroni's post hoc test using SPSS v22.0 (IBM Corp.). P <0.05 was considered to indicate a statistically significant difference.

Results

Metformin reduces human cervical cancer cell viability and migration. To determine the inhibitory effects of metformin, CaSki, C33A and HeLa cells were treated with various

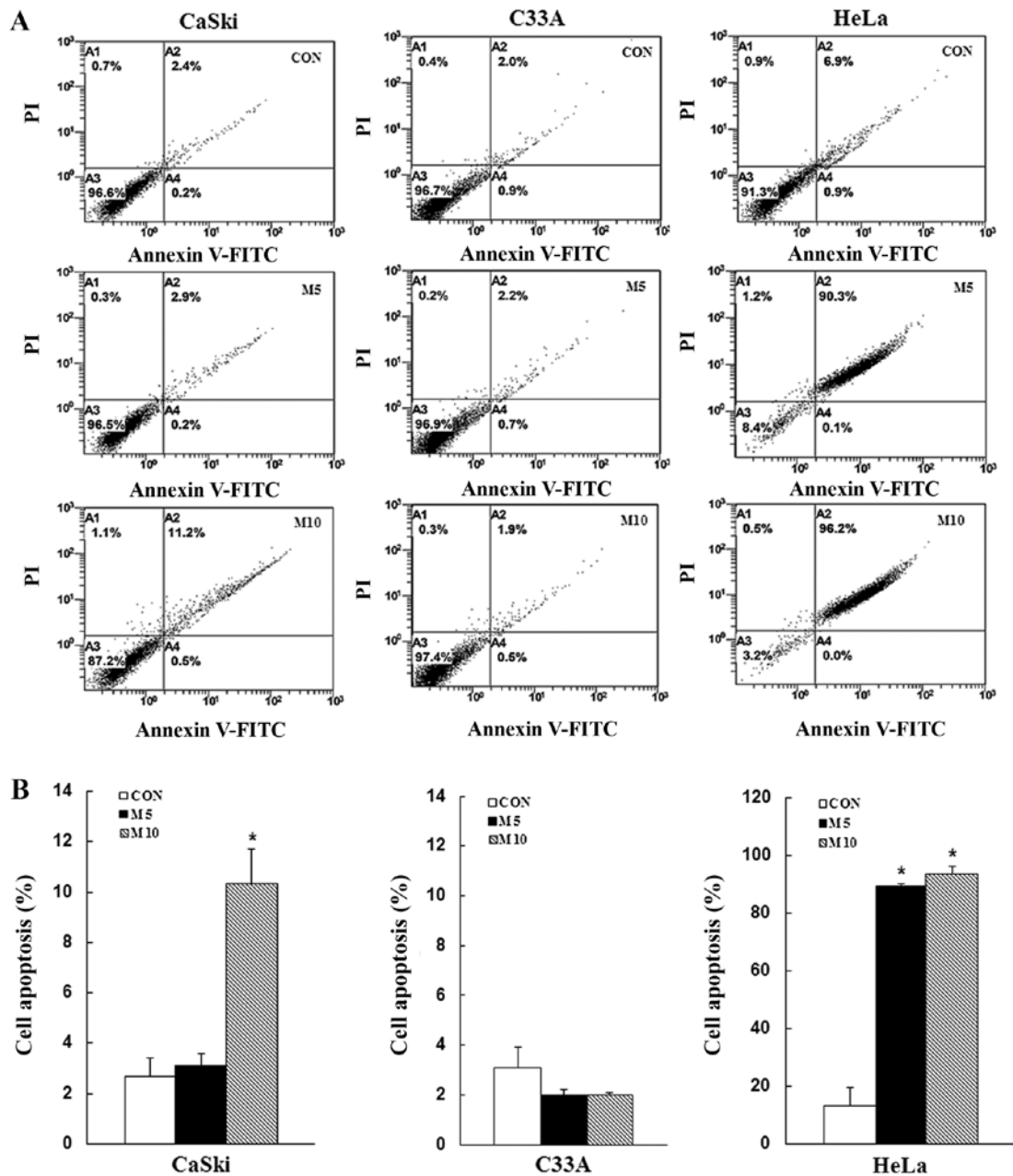


Figure 2. Metformin induces cervical cancer cell apoptosis. CaSki, C33A and HeLa cells were treated with metformin (0, 5 or 10 mM) for 48 h. Cell apoptosis was (A) determined via flow cytometry and (B) quantified. Data are presented as the mean \pm SD from three independent experiments. * $P < 0.05$ vs. CON. CON, 0 mM metformin; M5, 5 mM metformin; M10, 10 mM metformin.

concentrations of metformin for 48 h, and cell viability was determined using the CCK-8 assay. Compared with the control group, metformin significantly inhibited CaSki, C33A and HeLa cell viability in a dose-dependent manner (Fig. 1A). Similarly, compared with the control group, CaSki, C33A and HeLa cell migration was also markedly decreased following treatment with metformin at 48 h (Fig. 1B). The results suggested that metformin inhibited cervical cancer cell proliferation and migration.

Metformin induces apoptosis and cell cycle arrest in human cervical cancer cells. To determine whether the inhibitory effects of metformin on cell proliferation were mediated via induction of apoptosis and cell cycle arrest, CaSki, C33A and HeLa cells were stained with Annexin V/PI or PI/RNase,

respectively, and then analyzed via flow cytometry. Compared with the control group, metformin treatment increased the number of apoptotic cells in a dose-dependent manner (CaSki cells, 10.3 ± 1.1 vs. $2.7 \pm 0.7\%$; HeLa cells, 93.8 ± 2.5 vs. $13.3 \pm 6.6\%$), although no significant difference was observed in C33A cells (2.0 ± 0.1 vs. $3.1 \pm 0.8\%$) (Fig. 2). Furthermore, compared with the control group, metformin treatment significantly increased the number of CaSki and HeLa cells in the G_2/M phase, as well as the number of C33A and HeLa cells in the G_0/G_1 phase ($P < 0.05$; Fig. 3). The results indicated that metformin mediated cervical cancer cell death and proliferation via induction of apoptosis and cell cycle arrest.

Metformin activates the AMPK/p53 signaling pathway to mediate the mitochondrial apoptotic pathway. Due to its

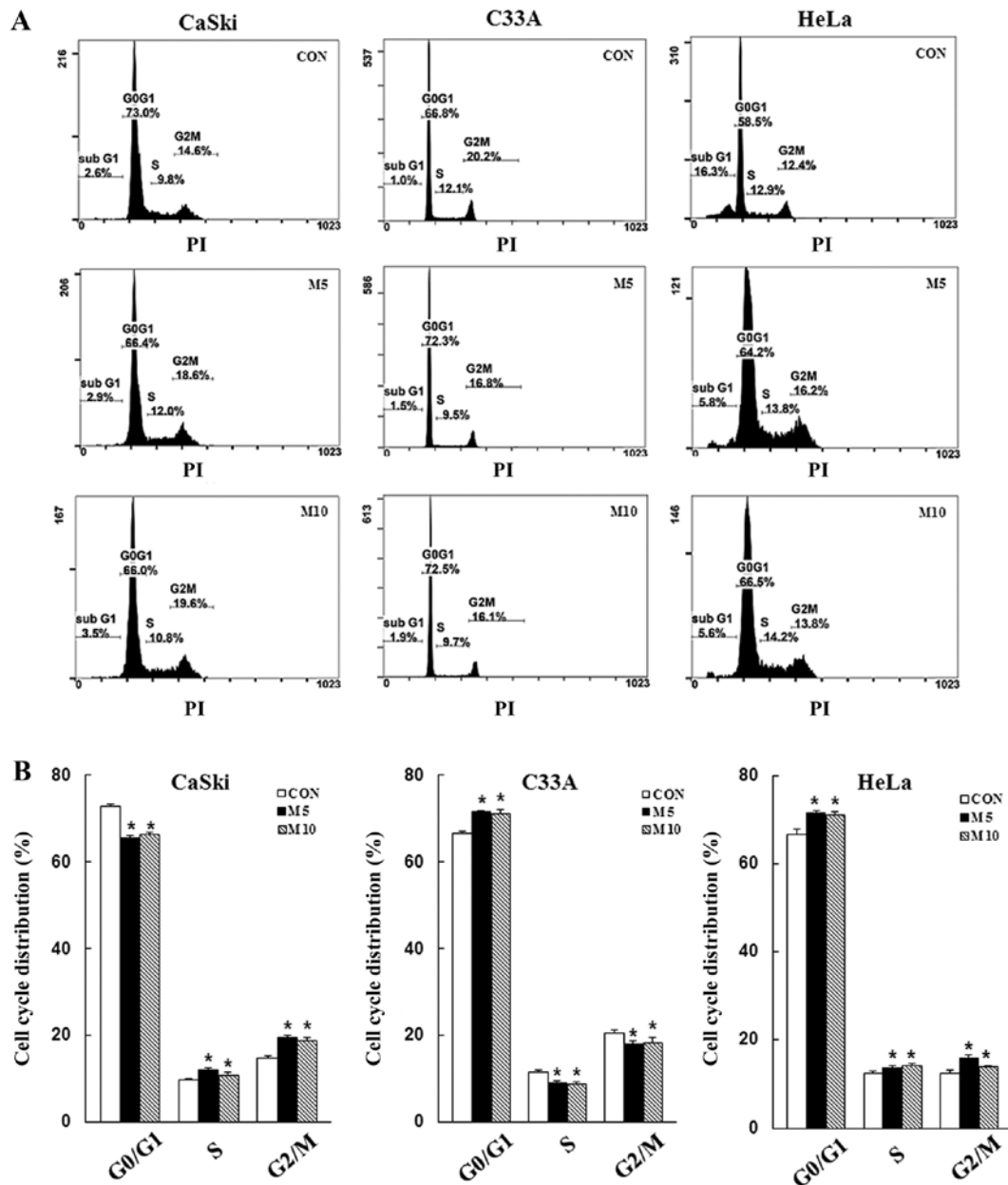


Figure 3. Metformin induces cervical cancer cell cycle arrest. CaSki, C33A and HeLa cells were treated with metformin (0, 5 or 10 mM) for 48 h. Cell cycle phase distribution was (A) determined via flow cytometry and (B) quantified. Data are presented as the mean \pm SD from three independent experiments. * P <0.05 vs. CON. CON, 0 mM metformin; M5, 5 mM metformin; M10, 10 mM metformin.

involvement in apoptotic regulation (41), AMPK/p53 signaling was investigated to determine whether metformin-induced AMPK and p53 activation contributed to apoptosis. In the present study, CaSki, C33A and HeLa cells were treated with 0, 5 or 10 mM metformin for 48 h, and the expression levels of AMPK, p53 and apoptosis-related proteins were determined via western blotting. In CaSki and C33A cells, compared with the control group, 5 mM metformin significantly increased the expression levels of p-AMPK, p-p53, Bak and Bax, and significantly decreased the expression levels of the antiapoptotic protein Bcl-2 (Fig. 4). By contrast, p-p53, Bak, Bax and Bcl-2 expression levels were significantly decreased in metformin-treated HeLa cells compared with control cells. Compared with the control group, high dose metformin (10 mM) treatment significantly increased the expression

levels of cleaved caspase-3 in CaSki, C33A and HeLa cells. The results indicated that metformin mediated apoptosis via targeting AMPK and mitochondria-mediated caspase-dependent signaling pathways.

Metformin decreases PI3K/AKT signaling. Previous studies have reported that the PI3K/AKT/mTOR signaling pathway is overactivated in numerous cancer types and its activation may promote cancer cell proliferation and migration, as well as inhibit apoptosis (42,43). In the present study, western blotting was performed to determine the expression levels of PIK3CA, p-AKT and p-p70S6K. Compared with the control group, 10 mM metformin significantly reduced PIK3CA, p-AKT and p-p70S6K expression levels in CaSki, C33A and HeLa cells (P <0.05; Fig. 5). The results suggested that the PI3K/AKT

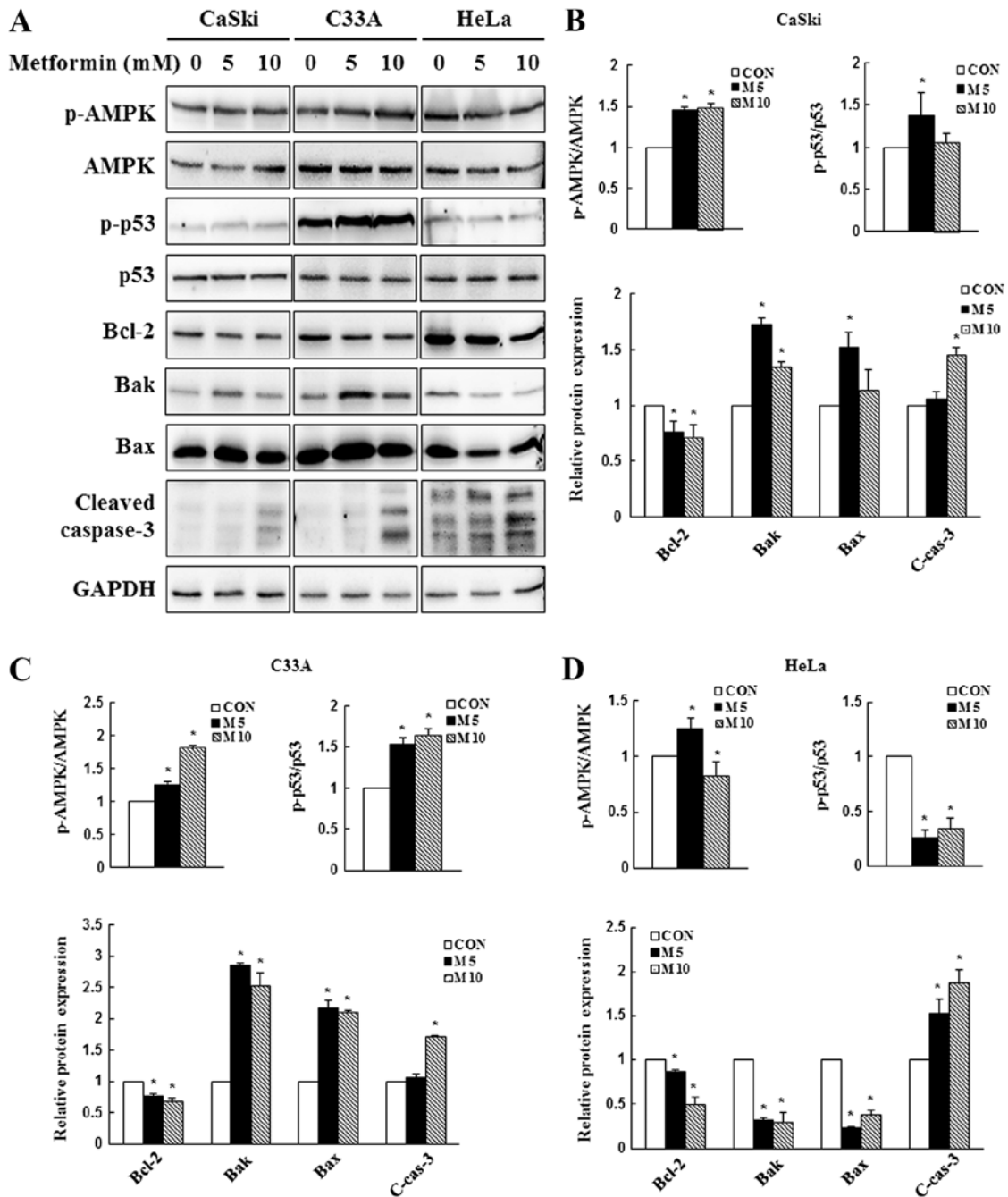


Figure 4. Effect of metformin on AMPK and caspase-dependent apoptosis signaling in cervical cancer cell lines. CaSki, C33A and HeLa cells were treated with metformin (0, 5 or 10 mM) for 48 h. p-AMPK, AMPK, p-p53, p53, Bcl-2, Bak, Bax and cleaved caspase-3 protein expression levels were (A) determined via western blotting and semi-quantified in (B) CaSki, (C) C33A and (D) HeLa cells. Data are presented as the mean \pm SD from three independent experiments. * $P < 0.05$ vs. CON. AMPK, AMP-activated protein kinase; p, phosphorylated; Bak, Bcl-2 antagonist/killer 1; CON, 0 mM metformin; M5, 5 mM metformin; M10, 10 mM metformin; C-cas-3, cleaved caspase-3.

signaling pathway might be involved in regulating cellular physiology and apoptosis following metformin treatment.

AMPK signaling pathway activity contributes to metformin-induced cytotoxicity and apoptosis in human cervical cancer cells. To assess whether the AMPK signaling pathway served a key molecular role in metformin-treated cervical cancer cells, CaSki, C33A and HeLa cells were pre-treated with or without 1 or 5 μ M Compound C for 2 h, and then treated with or without 10 mM metformin for 48 h. Cell viability and the expression levels of p-AMPK,

AMPK, p-p53, p53, Bcl-2 and cleaved caspase-3 were determined by performing the CCK-8 assay and western blotting, respectively. In CaSki and C33A cells, pre-treatment with Compound C significantly reversed the effects of metformin on cell viability, and suppressed p-AMPK, p-p53, cleaved caspase-3 and increased Bcl-2 expression levels compared with metformin treatment alone. By contrast, p-AMPK, p-p53 and Bcl-2 expression levels were significantly increased in the metformin + Compound C group compared with the metformin group in HeLa cells ($P < 0.05$; Fig. 6). The results indicated that metformin enhanced apoptosis by targeting

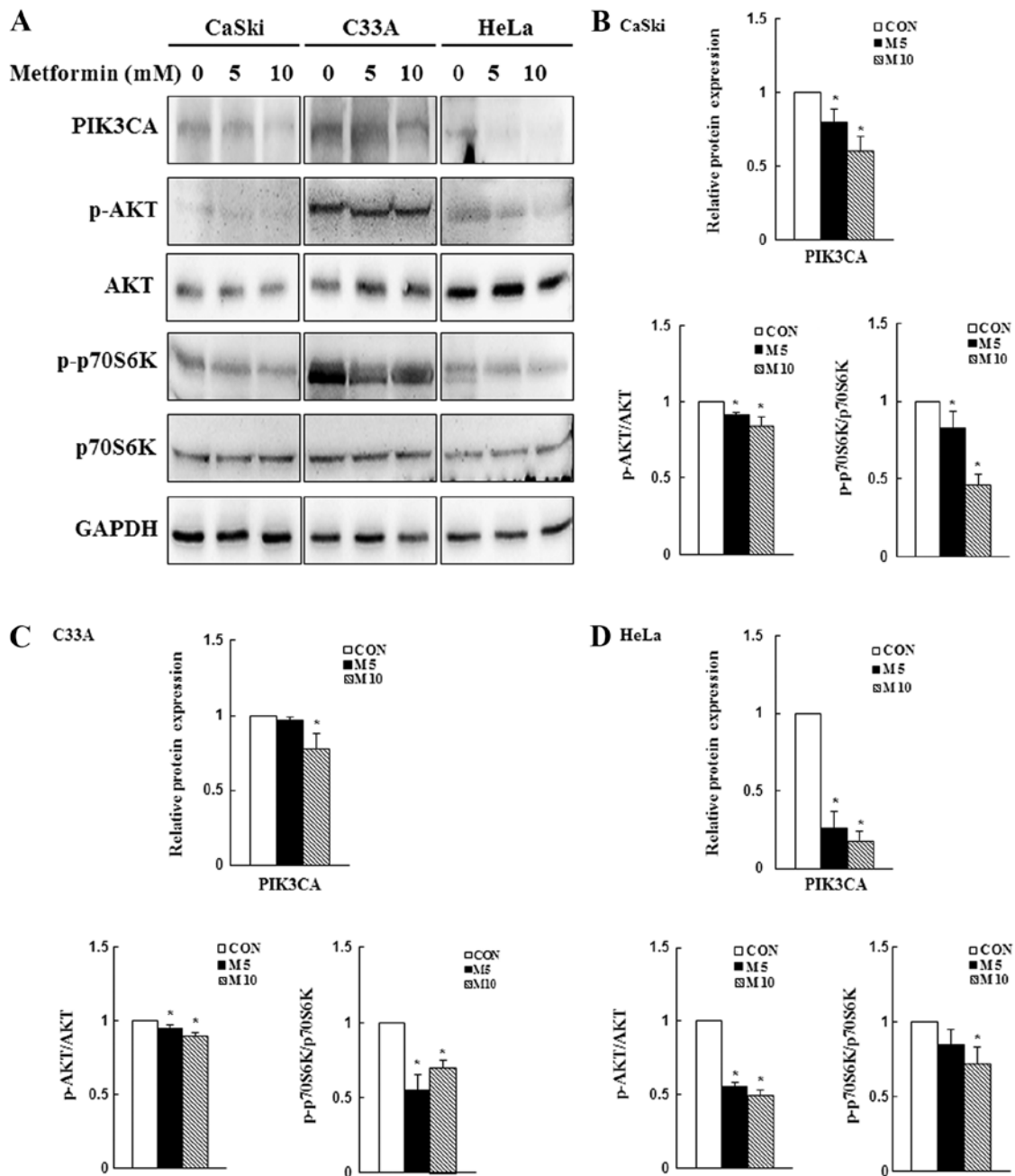


Figure 5. Effects of metformin on PI3K/AKT/mTOR signaling in cervical cancer cell lines. CaSki, C33A and HeLa cells were treated with metformin (0, 5 or 10 mM) for 48 h. Protein expression levels of PIK3CA, p-AKT, AKT, p-p70S6K, and p70S6K were (A) determined via western blotting and semi-quantified in (B) CaSki, (C) C33A and (D) HeLa cells. Data are presented as the mean \pm SD from three independent experiments. * $P < 0.05$ vs. CON. p, phosphorylated; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α ; p70S6K, p70S6 kinase; CON, 0 mM metformin; M5, 5 mM metformin; M10, 10 mM metformin.

the AMPK/p53 signaling pathway in CaSki, C33A and HeLa cells.

Discussion

Metformin is a widely used antidiabetic drug that has also been shown to reduce the risk of cancer and improve the efficacy of cancer treatment in patients with diabetes (44,45). However, multiple observational studies have demonstrated that metformin displays a similar protective effect and can improve the survival time of non-diabetic patients with cancer (46,47), but the results are controversial. In the present study, CCK-8,

Transwell and flow cytometry assays were performed to evaluate the effects of metformin on cell viability, migration, apoptosis and the cell cycle in human cervical cancer cells, respectively. Western blotting was conducted to quantify protein expression. The results of the present study indicated that metformin significantly reduced human cervical cancer cell viability (CaSki, C33A and HeLa) in a dose-dependent manner (Fig. 1A), which was consistent with previous studies that demonstrated that metformin significantly reduced the viability of thyroid cancer, osteosarcoma, leukemia and bile duct cancer cells by downregulating the expression of the antiapoptotic protein Bcl-2, and upregulating the expression

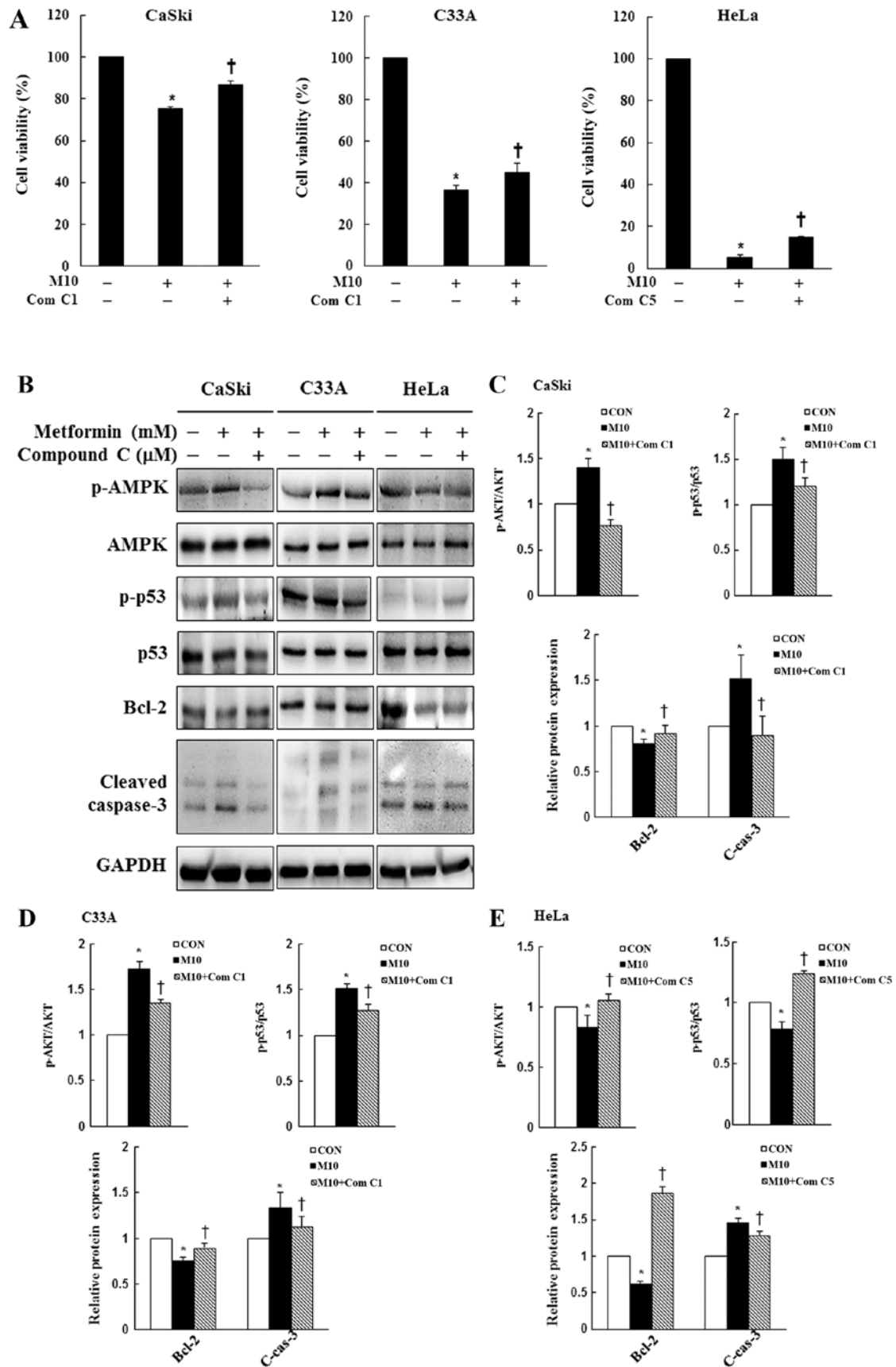


Figure 6. Effects of Compound C on cell viability, AMPK signaling and apoptotic signaling in cervical cancer cell lines. CaSki, C33A and HeLa cells were pre-treated with or without Compound C (1 or 5 μ M; an AMPK inhibitor) for 2 h and then treated with or without 10 mM metformin for 48 h, the concentration of metformin selected for experiment was based on the cell viability assay test. (A) Cell viability was determined by performing the Cell Counting Kit-8 assay. Protein expression levels of p-AMPK, AMPK, p-p53, p53, Bcl-2 and cleaved caspase-3 were (B) determined by western blotting and semi-quantified in (C) CaSki, (D) C33A and (E) HeLa cells. Data are presented as the mean \pm SD from three independent experiments. * P <0.05 vs. CON; † P <0.05 vs. M10. AMPK, AMP-activated protein kinase; p, phosphorylated; CON, 0 mM metformin; M10, 10 mM metformin; Com C1, 1 μ M Compound C; Com C5, 5 μ M Compound C; C-cas-3, cleaved caspase-3.

of the proapoptotic proteins Bak and Bax (16,48-50). The present study also indicated that HeLa cells were more sensitive to metformin compared with CaSki and C33A cells, which may be caused by cell-type specificity and mutations in cancer-related genes resulting in resistance to the anti-proliferative effects of metformin. CaSki cells, which are derived from small bowel metastasis, have been reported to possess an integrated human papilloma virus 16 genome (51). C33A cells are human papillomavirus (HPV)-negative human cervical cancer cells that display upregulated expression of oncogenes p53 and retinoblastoma protein (52). HeLa cells are a cervical adenocarcinoma-derived cell line containing integrated HPV-18 DNA and lower levels of p53 expression (53).

Consistent with the results of the present study, Hsieh Li *et al* (53) reported that metformin significantly induces apoptotic HeLa cell death and reduces p53 expression levels. By contrast, Xiao *et al* (54) reported that metformin is less effective against HeLa cells and enhances AMPK activation, but does not alter the expression levels of LKB1 or p53. Irie *et al* (55) also demonstrated that metformin is able to enhance LKB1 phosphorylation, promote AMPK and p53 activation, and inhibit cell cycle progression, resulting in cervical cancer cell death. Therefore, the mechanism underlying metformin-mediated effects on cervical cancer requires further investigation.

In addition, the present study indicated that metformin-induced cytotoxicity occurred via activation of the caspase-dependent apoptotic signaling pathway. Moreover, the results suggested that metformin induced cytotoxicity by promoting cell cycle arrest in the G₂/M phase and increasing apoptosis in CaSki and HeLa cells, and promoting G₀/G₁ phase cell cycle arrest in C33A and HeLa cells. Previous studies have illustrated the effects of metformin on the cell cycle in human osteosarcoma, demonstrating increased K7M2 and MG63 cell numbers in the G₀/G₁ phase, and increased U2OS and 143B cell numbers in the G₂/M phase (49,56). Additionally, metformin has been reported to enhance anticancer effects by arresting human colon carcinoma cells in the G₀/G₁ phase (SW480 cells) or the G₂/M phase (HCT116 p53^{-/-} cells) (57,58). The differences among cell lines may be associated with cancer cell line specificity and individual metformin bioavailability.

AMPK is not only a sensor of cellular energetics, but is also a crosstalk protein involved in apoptotic signaling pathways, including the LKB1-AMPK and AMPK/p53 signaling pathways (41,59). The results of the present study revealed that AMPK activation was consistent with increased p-p53 expression following metformin treatment, and that the AMPK inhibitor Compound C significantly alleviated p53 activation in metformin-treated cells. The aforementioned results indicated that the anticancer effect of metformin was mediated via the AMPK/p53 signaling pathway, although metformin did not induce p53 activation in HeLa cells. Furthermore, the results of the present study were consistent with previous reports that demonstrated that metformin induces the phosphorylation and activation of p53, which inhibits prostate, melanoma, lymphoma and acute myeloid leukemia cancer cell proliferation (49,60-62). Moreover, metformin can inhibit p53^{-/-} colorectal cancer cell proliferation both *in vitro* and *in vivo* (30). Previous studies have reported that metformin directly decreases the expression levels of endogenous p53

in sensitive cells, which downregulates the expression of target genes, including p21, Bax, Bak and BH3-only proteins (Bid and Bim), ultimately resulting in apoptosis (53,63,64). The results of the aforementioned previous studies were consistent with the results of the present study, which indicated that high-dose metformin (10 mM) induced CaSki cell apoptosis more effectively compared with low-dose metformin (5 mM), despite decreases in p-p53, Bak and Bax expression levels. Therefore, the present study indicated that metformin activated AMPK, inhibiting cervical cancer cell viability in both a p53-dependent and -independent manner.

Metformin displays inhibitory effects on cell proliferation, apoptosis, metastasis, angiogenesis and chemoresistance in various malignancies *in vitro* and *in vivo*, including ovarian (65), endometrial (66) and hepatocellular cancer (67). The inhibitory effects of metformin were mediated via activation of the PI3K/AKT/mTOR signaling pathway (65-67). In an earlier study, Storozhuk *et al* (68) also demonstrated that metformin inhibited tumor growth in non-small cell lung cancer (NSCLC) and xenograft animal models by activating the ATM serine/threonine kinase/AMPK/p53 signaling pathway and inhibiting the AKT/mTOR/eukaryotic translation initiation factor 4E-binding protein 1 signaling pathway, leading to an enhanced radiation response in NSCLC. Likewise, the clinical studies conducted by Dhillon *et al* (69) and Sayed *et al* (70) demonstrated that metformin treatment is associated with the improved overall survival of patients with NSCLC. In the present study, compared with the control group, metformin significantly decreased PIK3CA, p-AKT and p-p70S6K expression levels in CaSki, C33A and HeLa cells. The results indicated that metformin-mediated alterations to the PI3K/AKT/mTOR signaling pathway were associated with apoptosis induction in human cervical cancer cells.

In conclusion, the results of the present study suggested that metformin induced apoptosis and cell cycle arrest by modulating the AMPK/p53 and PI3K/AKT/mTOR signaling pathways. Collectively, the results indicated that metformin might serve as a novel therapeutic for human cervical cancer.

The present study had some limitations. First, it focused on the effects of metformin on cell death-related pathways in human cervical cancer cells but further studies should be conducted to elucidate the underlying molecular mechanism of the effects in human cervical cancer cells in more detail. Second, only human cervical cancer cell lines were used; future studies using animal models and patient tissues samples should be conducted to elucidate the mechanism underlying metformin-mediated effects on cervical cancer *in vivo*. Additional clinical studies are required to assess the safety and clinical efficiency of metformin for cervical cancer treatment.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Changhua Christian Hospital Research Foundation (grant nos. 108-CCH-IRP-132 and 108-CCH-IRP-059).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YHC and YHH designed the study, prepared the figures and drafted the manuscript. SFY and CKY designed the study. HDT, THC and MCC contributed to the conception of the work, drafted the manuscript and revised it critically for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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