doi.org/10.36721/PJPS.2023.36.1.REG.051-057.1

Angelicin inhibits the growth and migration of triple-negative breast cancer cells

Chen I-hsiao[1], Chen Chien-yu[2], Lan Yu-hsuan[3] and Chen I-fen[2*]
[1]Department of Medical Laboratory Science, I-Shou University, Kaohsiung, Taiwan
[2]Department of Biomedical Engineering, I-Shou University, Kaohsiung, Taiwan
[3]School of Pharmacy, China Medical University, Taichung, Taiwan

Abstract: Angelicin is a furocoumarin found in *Psoralea corylifolia* L. fruit and the Chinese herb *Angelica archangelica*. It exerts antitumor activities, including apoptosis, antiproliferation and anti-metastasis activities, in several types of cancers. However, its effects on human triple-negative breast cancer (TNBC) remain unclear. In this study, we evaluated the anticancer activity of angelicin *in vitro* in the TNBC cell line MDA-MB-231 and investigated the related molecular mechanisms. To determine the anticancer activity of angelicin, MTT assay and flow cytometric analysis were performed to measure the cytotoxicity, cell proliferation and cell cycle. Wound healing assay and trans well assay were used to analyze the migration and invasion of breast cancer cells. The effect of angelicin on the expression of proteins was analyzed by western blotting. The results revealed that angelicin (50, 100, 150μM) had no effect on cytotoxicity. However, angelicin (at 100 μM) could inhibit cell proliferation by reducing cyclin B1 and cdc2 and increasing p21 and p27 expression levels, thereby resulting in G2/M phase arrest. Additionally, angelicin at a concentration of 150 μM inhibited the migration and cell invasion of MDA-MB-231 cells, partially by downregulating MMP-2 protein levels. Together, these results suggest that angelicin may serve as an adjuvant chemotherapeutic agent for patients with TNBC.

Keywords: furocoumarin, angelicin, triple-negative breast cancer (TNBC), migration, bioactivity

INTRODUCTION

Cancer poses a substantial health and economic burden, requiring proactive procedures for prevention and treatment. Its incidence continues to rise and it is one of the main causes of mortality worldwide; the number of incident cases of cancer per year is estimated to reach 21 million by 2030. An efficient anticancer treatment is lacking (Ahmed *et al*., 2020; Ashraf, 2020; Singh *et al*., 2016). The high mortality of cancer is primarily because tumor cells have the abilities of locoregional invasion and distant metastases. Chemotherapy and radiotherapy are the main clinical approaches to cancer treatment, but both are associated with many adverse effects (Khan *et al*., 2020; Khattak and Khan, 2016; Rauf *et al*., 2015; Spector, 2018). Therefore, many studies have focused on effective anticancer therapy with minimal adverse effects.

Furanocoumarins are phytochemicals formed by the combination of coumarin and a furan ring. They have promising therapeutic potential in terms of anti-bacterial, antifungal, antiviral, anti-inflammatory, antiallergic, antioxidant and anticancer properties (Carbone *et al*., 2019; Olomola *et al*., 2014; Pynam and Dharmesh, 2018; Sumorek-Wiadro *et al*., 2020). The furanocoumarin angelicin is an active ingredient extracted from the plants of Leguminosae (Fabaceae), Apiaceae (Umbelliferae) and Moracea families (Kavli *et al*., 1983; Mahendra *et al*., 2020). It has anticancer, anti-inflammation, antiperiodontitis and pro-osteogenesis functions (Ge *et al*., 2019; Li *et al*., 2018; Wei *et al*., 2016). Angelicin suppresses tumor growth and malignant behavior in various human cancers, such as liver cancer (Wang *et al*., 2017), lung carcinoma A549 (Li *et al*., 2016), cervical carcinoma (HeLa), cervical squamous cell carcinoma (SiHa) (Wang *et al*., 2019), epithelioma (Hep2), colorectal carcinoma (HCT116), rhabdomyosarcoma (RD), breast adenocarcinoma (MCF-7) cell lines (Mira and Shimizu, 2015) and prostate cancer (PC-3) (Wang *et al*., 2015).

Breast cancer is the most common cancer and the leading cause of cancer mortality among women worldwide. Thus, many studies, particularly those on cancer immunotherapy, have focused on the role of the antitumor response in breast cancer (Schneider *et al*., 2008). Triple-negative breast cancer (TNBC) is a distinct pathological subtype of epithelial breast tumor that is immunohistochemically negative for the protein expression of estrogen receptor (ER) and progesterone receptor (PgR) and it does not overexpress human epidermal growth factor receptor 2 (HER2) (Carey *et al*., 2007; O'Reilly *et al*., 2015). Approximately, 15%-20% of invasive breast cancers are triple negative (Wang *et al*., 2022). In Taiwan, breast cancer has the highest incidence of all cancers (Chien *et al*., 2017; Wang *et al*., 2022). Patients with TNBC not only have a poor prognosis and high incidence of relapse and metastasis but also frequently develop chemoresistance, urgently necessitating novel therapeutic candidate drugs for TNBC (Hsieh *et al*., 2014; Marra *et al*., 2020).

In this study, we evaluated the effects of angelicin on the growth, migration and invasion in a breast cancer cell line to determine whether angelicin can serve as a promising compound for treating TNBC.

**Corresponding author:* e-mail: ifen@isu.edu.tw

MATERIALS AND METHODS

Materials

Angelicin was purchased from Chem Faces and dissolved in DMSO (Sigma, USA) to prepare a master stock solution at a concentration of 1 mg/mL and stored at -20°C before use. The working solution was freshly prepared from the complete culture medium.

Cell lines and culture conditions

Two human invasive TNBC cell lines, MDA-MB-231 and Hs 578T and mouse embryo fibroblasts, NIH-3T3, were obtained from American Type Tissue Culture Collection (ATCC, Manassas, VA, USA). The cells were grown in DMEM/F12 (Gibco; Thermo Fisher Scientific) supplemented with 100 µg/mL streptomycin, 100 U/mL penicillin and 10% FBS (Gibco) and maintained in a humidified incubator (NuAire) at 37°C and 5% CO_2. The cells were washed with phosphate-buffered saline (PBS) and subcultured as required with trypsin/EDTA (0.25%). The growth and morphology of each cell line were routinely monitored by light microscope.

MTT cytotoxic assay

Cell suspension (100 µL) at a density of 1.0×10^5 /well was seeded in a 96-well flat-bottomed plate. Following overnight adherence, the cells were treated with DMSO alone or with angelicin (50, 100 and 150µM) for 48 h. Next, MTT reduction assays were conducted according to a previously described method (Razak *et al*., 2019). For the proliferation assay, the cells were seeded in 96-well plates, allowed to adhere for 24h and then treated with various concentrations (0, 50, 100 and 150µM) of angelicin for 24, 48, 72 and 96h. Next, 20µL of MTT solution (Bio Basic, Canada) was added to each well and mixed. After 4 h, the supernatants were removed and 100µL of DMSO was added to each well to dissolve the precipitates. Cell viability was estimated by measuring absorbance at 570 nm on an ELISA plate reader (Bio-Tek Instruments, USA). The percentage of cell viability was calculated using the following formula: % Viability = (OD of treated cells/OD of control cells)×100. All the experiments were conducted in triplicate and in 3 independent tests.

Flow cytometry for cell cycle analysis

The breast cancer cells were treated with angelicin at 0, 50, 100 and 150 µM for 72h and fixed with 70% ethanol for 20 min. Both adherent and suspended cells were collected, centrifuged and washed twice with PBS. Thereafter, the cells were stained with 5µL of PI (2 mg/mL) with 0.5 µL of Tritone X-100 (0.1%) and 20µL of RNase A (4mg/mL) and then incubated for 30 min at room temperature. The cellular DNA content was analyzed using a FACS caliber cytometer (BD Accuri).

Monolayer wound-healing assay

The cells were seeded at a high density of 1×10^6 cells/well in 6-well plates and allowed to form a confluent monolayer. A vertical wound was created using a sterile 200 µL pipette tip across the cell layer. Next, the cells were incubated in complete growth medium (10% FBS) in the absence or presence of angelicin (0, 100, or 150µM) for 24h. The migration of the cells to the wound site was visualized under a microscope at 0, 5, 14 and 24h. The representative fields were photographed at 3 independent sites per well in 3 independent replicates. The ratio of cell migration was calculated as the percentage of the remaining cell-free area in the initial scratched area.

Transwell migration and invasion assays

Cell migration and invasion were assayed using transwell (BD Biosciences, USA), with PET track-etched membranes at the bottom with a pore size of 8 µm. For the invasion assay, the PET membranes were coated with a thin layer of Matrigel. In brief, 2×10^5 cells with or without angelicin treatment were resuspended in serum-free medium and transferred into the upper chamber of each trans well. The chemo attractant (10% FBS) was placed in each bottom well. After incubation for 24h, the cells remaining on the upper surface of the membrane were removed using a cotton swab. The migrated cells on the lower membrane surface were fixed with 100% methanol for 20 min and then stained with 0.5% crystal violet for 15 min. The stained cells were photographed and counted using a microscope (Nikon, Japan) at a magnification of 200×. Five randomly selected fields were photographed and counted per well.

Western blot analysis

The MDA-MB-231 cancer cells were treated with angelicin at 0, 50, 100, or 150µM for 24h, washed twice with cold PBS and lysed on ice in a lysis buffer containing protease inhibitors. The lysates were centrifuged at 12000 g for 15 min and the supernatant fractions were collected. Sample proteins (30µg/well) were separated using SDS-PAGE and transferred to nitrocellulose membranes. The membrane was blocked with 5% skim milk for 60 min and incubated with the primary antibody (β-Actin, 1:5000; p21, 1:1000; p27, 1:1000; MMP-2, 1:1000) overnight at 4°C. The membrane was incubated with HRP-conjugated secondary antibody at 4°C for 1h and then washed with TBST buffer. The immunoreactive bands were developed using a Super Signal West Pico Chemiluminescent Substrate kit and the molecular weight of the bands was confirmed.

STATISTICAL ANALYSIS

Data are presented as the mean ±standard deviation. The statistical significance of the difference among groups was assessed by student's t test using GraphPad Prism 5.0 software. $P<0.05$ was considered as statistically significant difference.

RESULTS

Cytotoxicity of angelicin on TNBC cells

The chemical structure of angelicin in presented in fig. 1A. To investigate the effects of angelicin in TNBC cells

(MDA-MB-231, Hs 578T) and comparison with non-cancer cell line (NIH-3T3), cells viabilities were determined using MTT assay following treatment with angelicin at the concentrations of 50, 100 and 150 μM for 48h. No cytotoxicity was observed in any of the cell lines. (fig. 1B).

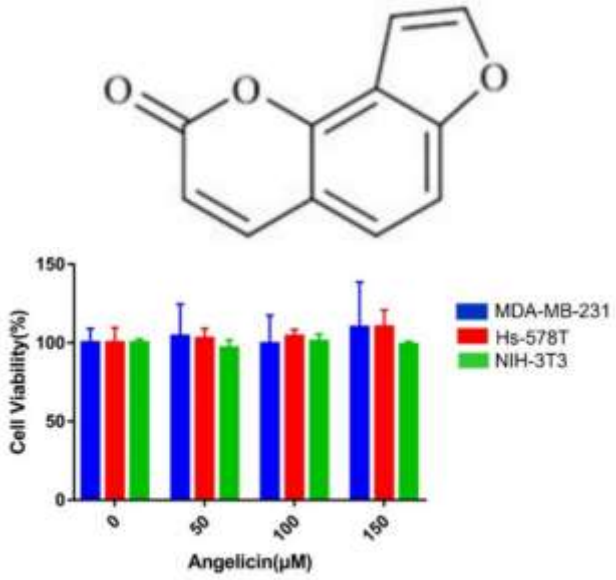

Fig. 1: Effect of angelicin on the cell viability of triple-negative breast cancer cells. (A) Chemical structure of angelicin. (B) Cell viability was measured by performing MTT assay following angelicin treatment (50,100,150 μM) for 48h. No cytotoxicity was observed in two TNBC cell lines (MDA-MB-231, Hs 578T) and mouse embryonic fibroblasts-NIH-3T3 cells.

Angelicin inhibited MDA-MB-231 cell proliferation through cell cycle arrest

Angelicin (100 μM) inhibited the growth of MDA-MB-231 cells (fig. 2). Compared with the control (0 μM), the number of living healthy cells decreased markedly after treatment with an increased concentration of angelicin for 72h compared with the control.

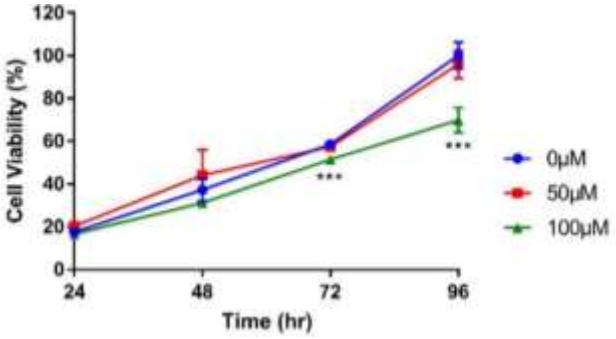

Fig. 2: Angelicin inhibits cell proliferation of MDA-MB-231 breast cancer cells. Cell proliferation was determined by MTT assay following treatment of angelicin (50,100μM) by the time course indicated. ***p<0.001 as compared with the control group (0μM).

Angelicin induced cell cycle arrest in the G2/M phase after 72-h treatment in a concentration-dependent manner (fig. 3). The ratio of cells in the G2/M phase following angelicin treatment increased significantly compared with control cells.

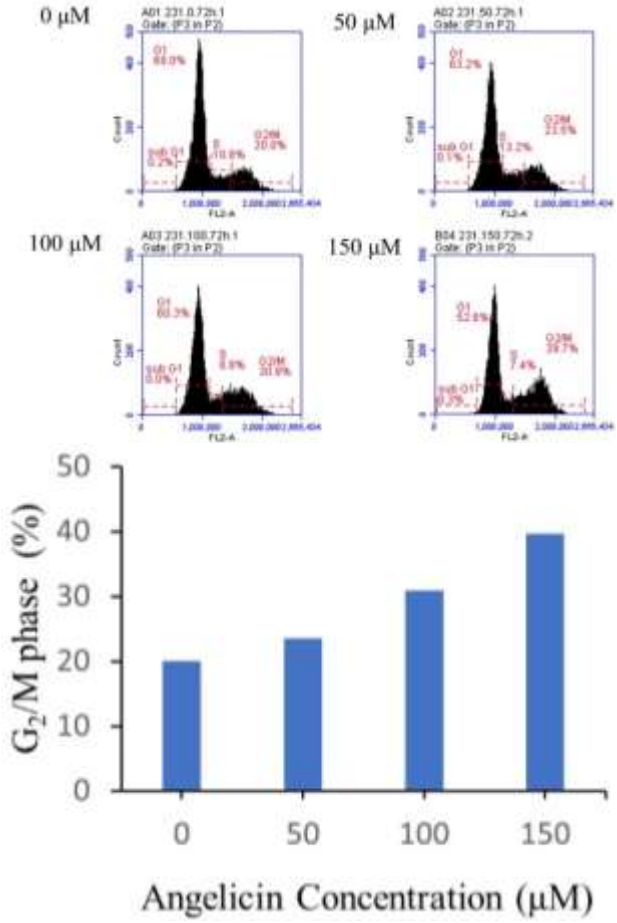

Fig. 3: Angelicin induces cell cycle arrest at the G2/M phase in MDA-MB-231 breast cancer cells. The cells were treated with angelicin at 0, 50, 100 and 150μM for 72h and cell cycle distribution was measured by flow cytometry following PI staining. Angelicin induced cell cycle arrest in the G2/M phase in a concentration-dependent manner.

To evaluate whether the regulatory proteins responsible for the cell cycle arrest, cyclin B1, cdc2 and cyclin-dependent kinase inhibitors (CKIs) participated in the angelicin-mediated G2/M phase arrest, p21 and p27 protein expression was investigated in MDA-MB-231 cells following 24- h treatment with angelicin. As shown in fig. 4, the levels of cyclin B1 and cdc2 were significantly down regulated in the angelicin-treated cells. A marked dose-dependent increase in the protein expression levels of p21 and p27 was observed following angelicin administration (fig. 4). These results suggest that angelicin inhibits MDA-MB-231 cell proliferation through G2/M phase arrest, at least partially by reducing cyclin B1 and cdc2 and increasing p21 and p27 protein levels.

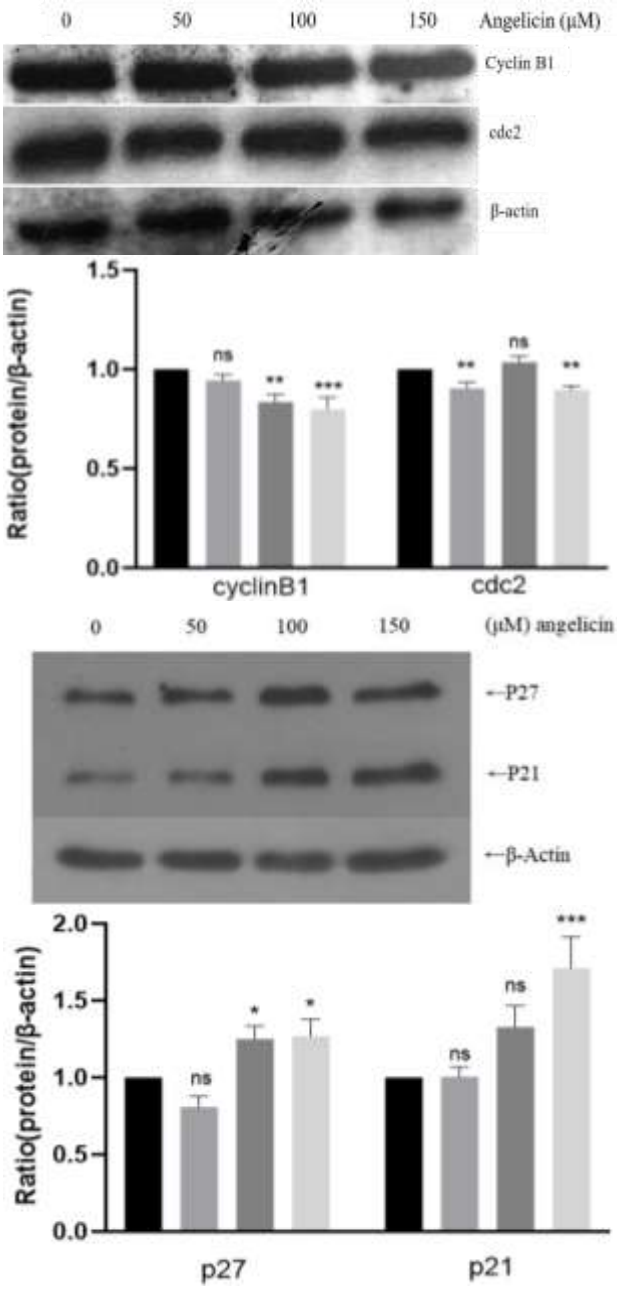

Fig 4: Effect of Angelicin on cell cycle protein expression of cyclin B1, cdc2, p21 and p27 by western blotting. (A) The MDA-MB-231 cells were treated with angelicin at different concentrations (0, 50,100,150μM) for 24h and expression of proteins was analyzed. β-actin was used as an internal control. (B) Quantitation data of (A). Columns, means of 3 independent experiments; bars, SD. ns, nonsignificant; *P<0.05, **P<0.01, ***P<0.001 compared with DMSO (0μM)

Angelicin inhibited MDA-MB-231 cell mobility

To evaluate the biological function of angelicin in MDA-MB-231 cells, we performed wound-healing and transwell migration and invasion assays. Scratch distances and width closure were obtained through software comparison between images from 0 to 24h. The results revealed that 150 μM angelicin, but not 100 μM angelicin, suppressed the migration of MDA-MB-231 cells compared with mock-treated cells (fig. 5). In line with the wound- healing assay, transwell assays revealed that angelicin inhibited the migration (fig. 6A) and invasion (fig. 6B) of MDA-MB-231 cells.

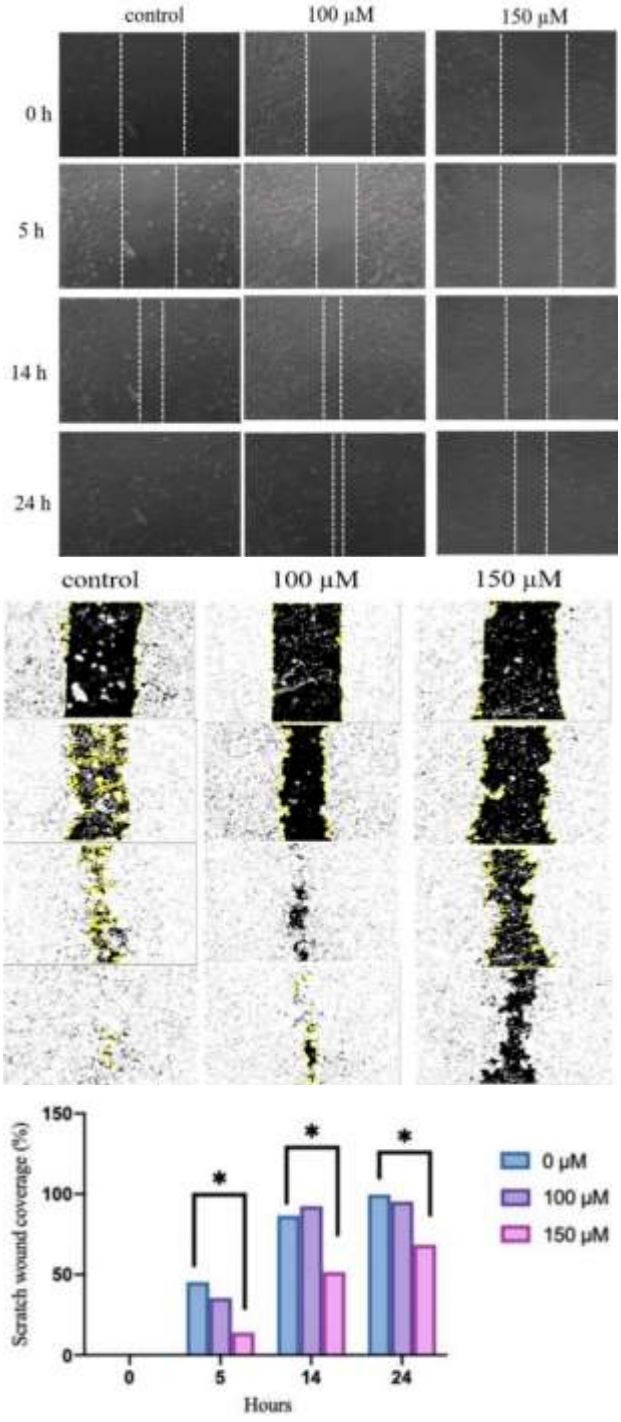

Fig. 5: Angelicin inhibits cell mobility of MDA-MB-231 cells by wound healing assay. (A) Confluent monolayers of MDA-MB-231 cells were scratched with a pipette tip,

then treated with DMSO (control) or Angelicin (100, 150 µM) and recovering of wounded areas (marked by white lines) at different time points. Images were recorded using an inverted microscope at indicated periods. (B) Dark field is defined the areas lacking cells (wound area, Image J software) and the percentage of scratch wound coverage was determined by Image J software (C). *P<0.05 vs. control group.

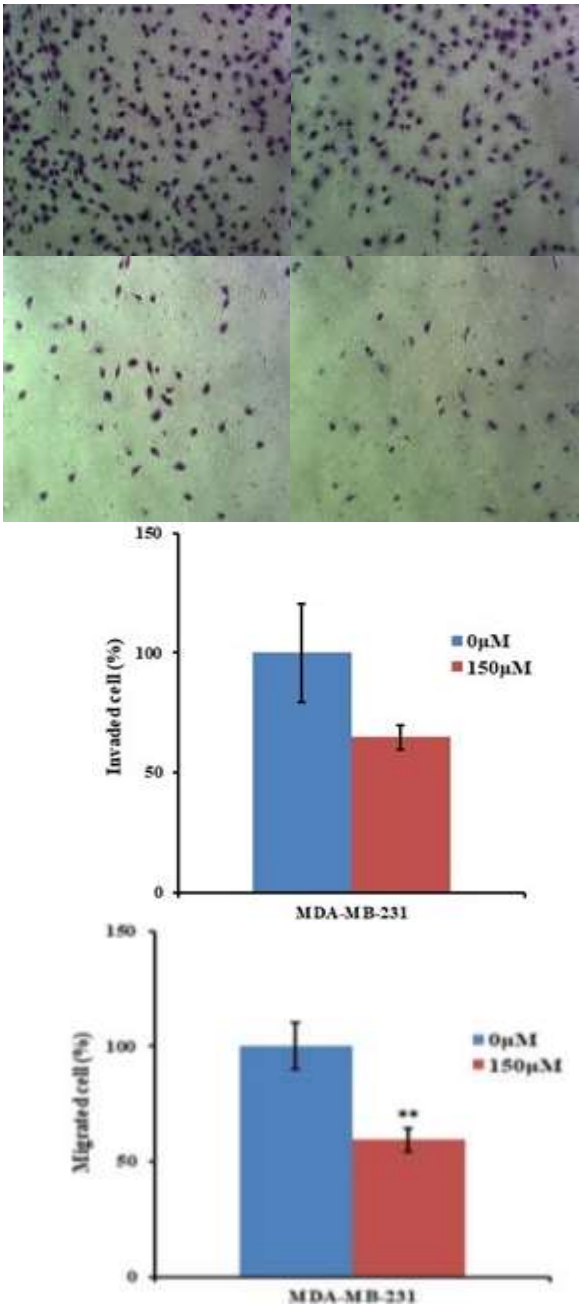

Fig. 6: Angelicin inhibits cell mobility *in vitro*. The MDA-MB-231 cells were treated with angelicin at 150µM for 24h and cell migration (A) and invasion ability (B) were analyzed using trans well assays. The bar graph represents the results of 3 independent experiments. **P<0.01 vs. the control group.

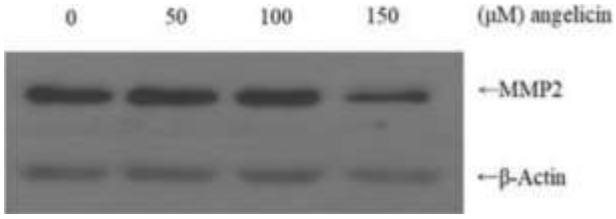

Fig. 7: Angelicin decreases the expression of MMP-2 in MDA-MB-231 cells. The cells were treated with angelicin at different concentrations (0, 50, 100 and 150 µM) for 24 h and the level of MMP-2 expression was determined by western blot analysis. β-actin was used as an internal control.

Angelicin down regulated MMP-2 expression in MDA-MB-231 cells

MMP-2 and MMP-9 are important for cancer cell migration and invasion (Lemieux *et al*., 2009). Thus, in this study, we determined the effect of angelicin on MMP-2 expression in MDA-MB-231 breast cancer cells. Angelicin (0, 50, 100, or 150 µM) was added to the cells, followed by incubation for 24h. As presented in fig. 7, 150µM angelicin significantly decreased MMP-2 expression in MDA-MB-231 cells compared with control cells.

DISCUSSION

The furanocoumarin angelicin has been reported to possess anticancer and antitumor properties (Ahmed *et al*., 2020; Mahendra *et al*., 2020). In liver cancer, angelicin treatment induces mitochondrial-dependent apoptosis (Wang *et al*., 2017). In the non-small cell lung cancer cell line A549 and its multidrug-resistant cell line A549/D16, angelicin promoted chemotherapy-induced apoptosis (Hsieh *et al*., 2014). It also induces A549 cell apoptosis by inducing caspase-3 and caspase-9 activation and increasing the ratio of Bax to Bcl-2 (Li *et al*., 2016). It has also been demonstrated to induce cervical cancer cell apoptosis (Wang *et al*., 2019). In breast cancer, studies evaluating the anticancer effects of angelicin and the underlying mechanisms thereof have focused on ER positive cancer cell line MCF-7 (Acharya *et al*., 2019; Mira and Shimizu, 2015). Therefore, in the present study, we assessed its anticancer effects on the TNBC cell line MDA-MB-231 and the possible molecular mechanisms underlying its action.

In this study, angelicin (50, 100, 150 µM) was not cytotoxic to TNBC cell lines (MDA-MB-231 and Hs-578T) (fig. 1B) but inhibited the proliferation of MDA-MB-231 cells at 100µM after 72h treatment (fig. 2). Cell cycle dysregulation contributes to cancer cell growth; thus, cell cycle arrest is considered effective in inhibiting cancer cell proliferation. Angelicin induced cell cycle arrest in the G2/M phase after 72-h treatment in a dose-dependent manner (fig. 3). Studies have demonstrated that angelicin can inhibit lung carcinoma A549 cell growth through

G2/M phase arrest and cervical cancer cell proliferation through G1 phase arrest (Li *et al*., 2016; Wang *et al*., 2019). Additionally, cell cycle progression is closely related to various cyclins, cyclin-dependent kinases and CKIs. We observed that cyclin B1, cdc2, p21 and p27, key regulators of the cell cycle, were modulated by angelicin (fig. 4). These results indicate that angelicin could retard cell proliferation by regulating cyclin B1, cdc2, p21 and p27 protein levels, thereby arresting the cell cycle in the G2/M phase.

Tumor metastasis is a major cause of death in patients with cancer. In this study, we observed that 150 μM angelicin significantly inhibited MDA-MB-231 cell migration and invasion compared with mock-treated cells after 24h treatment (fig. 5 and 6), whereas no effect on cell viability was observed after 24h treatment of angelicin at 150μM (fig. 1). The results agreed well with reports that angelicin significantly inhibits the migration and invasion of cervical cancer cells (Wang *et al*., 2019) and non-small cell lung carcinoma cell line A549 by regulating the JNK and ERK pathways (Li *et al*., 2016). MMP-2 and MMP-9 expression levels play key roles in regulating cancer cell invasion and metastasis (Lemieux *et al*., 2009). Our results revealed that 150 μM angelicin markedly decreased MMP-2 expression after 24h treatment (fig. 7). Overexpression of MMPs in tumor cells enhances their migration and invasion abilities, thus promoting cancer metastasis (Qin *et al*., 2008). Therefore, angelicin inhibited MDA-MB-231 cell invasion *in vitro* at least partly by down regulating MMP-2.

CONCLUSION

We demonstrated that angelicin exerted anticancer activity by arresting the cell cycle in the G2/M phase and by inhibiting the migration and invasion of human TNBC MDA-MB-231 cells. The mechanisms underlying these effects are associated with decreased cyclin B1, cdc2, increased p21, p27 and down regulated MMP-2; however, further studies are required to elucidate the exact underlying molecular mechanisms. Together, our data imply that angelicin is a potential adjuvant therapeutic agent against TNBC.

ACKNOWLEDGMENTS

This research was funded by Minister of Science and Technology (107-2813-C-214-068-B). This manuscript was edited by Wallace Academic Editing.

REFERENCES

Acharya R, Chacko S, Bose P, Lapenna A and Pattanayak SP (2019). Structure based multitargeted molecular docking analysis of selected furanocoumarins against breast cancer. *Sci. Rep.*, **9**(1): 15743.

Ahmed S, Khan H, Aschner M, Mirzae H, Kupeli Akkol E and Capasso R (2020). Anticancer potential of furanocoumarins: Mechanistic and therapeutic aspects. *Int. J. Mol. Sci.*, **21**(16): 5622-5642.

Ashraf MA (2020). Phytochemicals as potential anticancer drugs: time to ponder nature's bounty. *Biomed. Res. Int.*, **2020**: 8602879.

Carbone A, Montalbano A, Spanò V, Musante I, Galietta LJV and Barraja P (2019). Furocoumarins as multi-target agents in the treatment of cystic fibrosis. *Eur. J. Med. Chem.*, **180**: 283-290.

Carey LA, Dees EC, Sawyer L, Gatti L, Moore DT, Collichio F, Ollila DW, Sartor CI, Graham ML and Perou CM (2007). The triple negative paradox: Primary tumor chemosensitivity of breast cancer subtypes. *Clin. Cancer Res.*, **13**(8): 2329-2334.

Chien LH, Tseng TJ, Chen CH, Jiang HF, Tsai FY, Liu TW, Hsiung CA and Chang IS (2017). Comparison of annual percentage change in breast cancer incidence rate between Taiwan and the United States A smoothed Lexis diagram approach. *Cancer Med.*, **6**(7): 1762-1775.

Ge L, Cui Y, Liu B, Yin X, Pang J and Han J (2019). ERα and Wnt/β-catenin signaling pathways are involved in angelicin-dependent promotion of osteogenesis. *Mol. Med. Rep.*, **19**(5): 3469-3476.

Hsieh MJ, Chen MK, Yu YY, Sheu GT and Chiou HL (2014). Psoralen reverses docetaxel-induced multidrug resistance in A549/D16 human lung cancer cells lines. *Phytomedicine*, **21**(7): 970-977.

Kavli G, Midelfart K, Raa J and Volden G (1983). Phototoxicity from furocoumarins (psoralens) of *Heracleum laciniatum* in a patient with vitiligo. Action spectrum studies on bergapten, pimpinellin, angelicin and sphondin. *Contact Derm.*, **9**(5): 364-366.

Khan H, Reale M, Ullah H, Sureda A, Tejada S, Wang Y, Zhang ZJ and Xiao J (2020). Anti-cancer effects of polyphenols via targeting p53 signaling pathway: Updates and future directions. *Biotechnol. Adv.*, **38**: 107385.

Khattak S and Khan H (2016). Anti-cancer potential of phyto-alkaloids: A prospective review. *Curr. Cancer Ther. Rev.*, **12**(1): 66-75.

Lemieux E, Bergeron S, Durand V, Asselin C, Saucier C and Rivard N (2009). Constitutively active MEK1 is sufficient to induce epithelial-to-mesenchymal transition in intestinal epithelial cells and to promote tumor invasion and metastasis. *Int. J. Cancer*, **125**(7): 1575-1586.

Li G, He Y, Yao J, Huang C, Song X, Deng Y, Xie S, Ren J, Jin M and Liu H (2016). Angelicin inhibits human lung carcinoma A549 cell growth and migration through regulating JNK and ERK pathways. *Oncol. Rep.*, **36**(6): 3504-3512.

Li X, Yu C, Hu Y, Xia X, Liao Y, Zhang J, Chen H, Lu W, Zhou W and Song Z (2018). New application of psoralen and angelicin on periodontitis with anti-bacterial, anti-

inflammatory and osteogenesis effects. *Front Cell Infect. Microbiol.*, **8**: 178.

Mahendra CK, Tan LTH, Lee WL, Yap WH, Pusparajah P, Low LE, Tang SY, Chan KG, Lee LH and Goh BH (2020). Angelicin-a furocoumarin compound with vast biological potential. *Front Pharmacol.*, **11**: 366.

Marra A, Trapani D, Viale G, Criscitiello C and Curigliano G (2020). Practical classification of triple-negative breast cancer: Intratumoral heterogeneity, mechanisms of drug resistance and novel therapies. *NPJ Breast Cancer*, **6**: 54.

Mira A and Shimizu K (2015). *In vitro* cytotoxic activities and molecular mechanisms of *Angelica shikokiana* extract and its isolated compounds. *Pharmacogn. Mag.*, **11**(Suppl 4): S564-S569.

Olomola TO, Mosebi S, Klein R, Traut-Johnstone T, Coates J, Hewer R and Kaye PT (2014). Novel furocoumarins as potential HIV-1 integrase inhibitors. *Bioorg. Chem.*, **57**: 1-4.

O'Reilly EA, Gubbins L, Sharma S, Tully R, Guang MH, Weiner-Gorzel K, McCaffrey J, Harrison M, Furlong F, Kell M and McCann A (2015). The fate of chemoresistance in triple negative breast cancer (TNBC). *BBA Clin.*, **3**: 257-275.

Pynam H and Dharmesh SM (2018). Antioxidant and anti-inflammatory properties of marmelosin from Bael (*Aegle marmelos* L.); Inhibition of TNF-α mediated inflammatory/tumor markers. *Biomed. Pharmacother.*, **106**: 98-108.

Qin L, Liao L, Redmond A, Young L, Yuan Y, Chen H, O'Malley BW and Xu J (2008). The AIB1 oncogene promotes breast cancer metastasis by activation of PEA3-mediated matrix metalloproteinase 2 (MMP-2) and MMP-9 expression. *Mol. Cell Biol.*, **28**(19): 5937-5950.

Rauf A, Khan R, Khan H and Tokuda H (2015). Cytotoxic, antitumor-promoting and inhibition of protein denaturation effects of flavonoids, isolated from *Potentilla evestita* Th. Wolf. *Nat. Prod. Res.*, **29**(18): 1775-1778.

Razak NA, Abu N, Ho WY, Zamberi NR, Tan SW, Alitheen NB, Long K and Yeap SK (2019). Cytotoxicity of eupatorin in MCF-7 and MDA-MB-231 human breast cancer cells via cell cycle arrest, anti-angiogenesis and induction of apoptosis. *Sci. Rep.*, **9**(1): 1514.

Schneider BP, Winer EP, Foulkes WD, Garber J, Perou CM, Richardson A. Sledge GW and Carey LA (2008). Triple-negative breast cancer: Risk factors to potential targets. *Clin. Cancer Res.*, **14**(24): 8010-8018.

Singh S, Sharma B, Kanwar SS and Kumar A (2016). Lead phytochemicals for anticancer drug development. *Front. Plant. Sci.*, **7**: 1667.

Spector D (2018). Optimizing cancer survivors' health: The role of lifestyle behaviors. *J. Nurse Pract.*, **14**(4):323-329.

Sumorek-Wiadro J, Zając A, Maciejczyk A, Jakubowicz-Gil J, Joanna SW, Adrian Z, Aleksandra M and Joanna JG (2020). Furanocoumarins in anticancer therapy-For and against. *Fitoterapia*, **142**: 104492.

Wang F, Li J, Li R, Pan G, Bai M and Huang Q (2017). Angelicin inhibits liver cancer growth *in vitro* and *in vivo*. *Mol. Med. Rep*., **16**(4): 5441-5449.

Wang Q, Wang YP, Lin H, Zhang LQ, Wu LJ and Pang LX (2015). Antiproliferative and apoptotic effects of angelicin in highly invasive prostate cancer cells. *Trop. J. Pharm. Res.*, **14**(8): 1405-1411.

Wang Y, Chen Y, Chen X, Liang Y, Yang D, Dong J, Yang N and Liang Z (2019). Angelicin inhibits the malignant behaviours of human cervical cancer potentially via inhibiting autophagy. *Exp. Ther. Med.*, **18**(5): 3365-3374.

Wang YC, Wang ZH, Yen JH, Shen YC, Shen TC, Chang WS, Su CH, Chen KY, Yen CM, Lee HT, Yang JS, Bau DT and Tsai CW (2022). The contribution of interleukin-8 Rs4073 genotypes to triple negative breast cancer risk in Taiwan. *Anticancer Res.*, **42**(8): 3799-3806.

Wei DZ, Guo XY, Lin LN, Lin MX, Gong YQ, Ying BY and Huang MY (2016). Effects of angelicin on ovalbumin (OVA)-induced airway inflammation in a mouse model of asthma. *Inflammation*, **39**(6): 1876-1882.