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Cycloartobiloxanthone Inhibits Migration and Invasion of Lung Cancer Cells

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Abstract. *Background/Aim: Metastasis in lung cancer is a major cause of high mortality. Metastasis depends on the potential of cancer cells to migrate and invade. Here we demonstrated the anti-migration and invasion activities of the compound cycloartobiloxanthone from Artocarpus gomezianus Wall. ex Tréc. (Moraceae). Materials and Methods: The effect of the compound on viability of human lung cancer H460 cells was investigated by 3-[4,5-dimethylthiazol-2-yl]-2,5diphenyl tetrazoliumbromide (MTT) assay. Migration and invasion assays were performed. Filopodia formation was determined by phalloidin-rhodamine staining. The hallmark signaling proteins in regulation of epithelial to mesenchymal transition (EMT), migration, and integrin α5, αV, β1 and β3 were determined by western blot analysis. Results: Cycloartobiloxanthone at concentrations lower than 10 μM has no cytotoxic effects. Regarding cell motility, cycloartobiloxanthone at 5-10 μM and 1-10 μM exhibited anti-migration and anti-invasion activities, respectively. Filopodia were found to be significantly reduced in cycloartobiloxanthone-treated cells. These effects correlated with the results from western blot analysis showing that the phosphorylation of focal adhesion kinase on Try397 (p-FAK (Try397)), and cell division cycle 42 (CDC42) were significantly reduced. Cycloartobiloxanthone significantly suppressed migratory integrins including integrin α5, αV, and β3, while had no significant effect on integrin β1. Besides, the compound suppressed epithelial to mesenchymal transition in lung cancer H460 cells indicated by the change in cell morphology form fibroblast-like to epithelial morphology with up-regulation of E-cadherin. Conclusion: Cycloartobiloxanthone possesses anti-migration and anti-invasion properties by suppressing several migratory-regulated mechanisms including suppressing migratory FAK and CDC42 signal, reduced filopodia of migrating cells, decreasing integrin α5, αv and β3, and inhibiting EMT. Our findings demonstrated the potentials of cycloartobiloxanthone for further studies and developments.*

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Lung cancer is highly prevalent and aggressive cancer that recognized as the leading cause of cancer-related death worldwide (1). Lung cancer is a characterized by uncontrolled cell growth in tissues of the lung and can spread by the process of metastasis into other parts of the body. Human lung cancers are classified into two major types, small cell lung cancer (SCLC) and non-small cell lung carcinoma (NSCLC) (2). Especially, non-small cell lung cancer (NSCLC) represents 75-80% of all lung carcinomas (3) and has overall 5-year survival rate only 15% (4).

Cancer metastasis is one major cause of high rates of death in lung cancer (5). Metastasis involves ability of the cells to migrate form their origin and invaded surrounding tissue for entering blood and lymphatic circulations (6, 7). So far, the ability of cancer cells to migrate and invade is an important facilitating factors of successful cancer cell metastasis (8).

During the process of cell movement, the transmembrane receptors called integrins providing links of extracellular matrix (ECM) to the intracellular actin cytoskeleton at the points of cell–substratum interaction (focal adhesions), initiate cellular signals that promote cell migration (9). Focal adhesion kinase (FAK) is a protein recruited to sites of integrin clustering and initiates signaling pathways in the

control of cell motility and invasion (9). Activated FAK can activate the signal through the phosphorylation of protein kinase B (AKT) resulting in cell invasion and migration (10). Likewise, recent research found that phosphorylated Akt contributes to the up-regulation of the Rho family especially cell division cycle (Cdc42) that has been shown to play an essential role in actin reorganization and filopodia formation (11). Additionally, recent studies have extended the knowledge of epithelial mesenchymal-transition (EMT) because this process is involved in metastasis (12). In this process, epithelial cells lose cell-cell adhesion and cell polarity. E-cadherin, the epithelial cells marker is considered a suppressor of tumor invasion. Therefore, the loss of E-cadherin has been associated with metastatic dissemination (13).

Cycloartobiloxanthone is a natural compound obtained from *Artocarpus gomezianus* Wall. ex Tréc. (Moraceae), and is known as “Hat-nun” or “Ka-Noon-Pah” in Thailand. Cycloartobiloxanthone was reported to have an identifiable DPPH free radical scavenging ability and inhibitory effect on nitric oxide production in murine macrophage-like cells (14). It was also discovered in the root barks of *Artocarpus altilis*. Cycloartobiloxanthone showed antitubercular and antiplasmodial activities, and showed cytotoxicity against human oral epidermoid carcinoma and human breast cancer (15). However, the effects of cycloartobiloxanthone on cancer cell migration and invasion are unknown. This study aims to investigate the effects of cycloartobiloxanthone on cancer cell migration and invasion to understand the underlying mechanisms which would benefit to the development of anti-metastasis drugs.

Materials and Methods

Test compound. Cycloartobiloxanthone was isolated from *Artocarpus gomezianus* Wall. ex Tréc. (Moraceae) as previous described (14). Its purity was determined using HPLC and NMR spectroscopy. It was dissolved in DMSO and RPMI-1640 in order to achieve the desired concentrations, which all contained less than 0.2% DMSO at final dilution.

Cell culture. Human lung cancer H460 cells were obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin/streptomycin and 2 mM L-glutamine, the cells were incubated in a 5% CO_2 environment at 37˚C.

Chemicals. Propidium iodide (PI) and Hoechst 33342 were obtained from Molecular Probes, Inc. (Eugene, OR). 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and phalloidin tetramethylrhodamine B isothiocyanate were obtained from Sigma Chemical, Inc. (St. Louis, MO, USA). Antibody for p-Akt, p473-Akt, FAK, p397-FAK, Cdc42, Integrin αV, Integrin α5, Integrin β1, Integrin β3, E-cadherin and b-actin, as well as peroxidase-conjugated secondary antibodies were obtained from Cell Signaling Technology, Inc. (Danvers, MA, USA).

Cytotoxicity assay. To determine cycloartobiloxanthone-mediated cytotoxicity, cell viability was determined by the MTT assay. After treatments, the cells were incubated with 0-100 μM MTT solution for 4 h at 37˚C. An intensity reading of the MTT product was measured at 570 nm using a microplate reader, and the percentage of viable cells was calculated in relation to control cells. All analyses were performed in 3 independent replicate cell cultures.

Migration assay. Migration was determined by wound healing assays. A monolayer of cells was cultured in 96-well plates. The wound space was created by 1-mm-wide tips. After that, media was removed and washed with PBS. The cell monolayers were incubated with non-toxic concentration of cycloartobiloxanthone (0-10 μM) and permitted to migrate for 24 h, 48h. Under a phase contrast microscope (Olympus DP70, Melville, NY, USA), Micrographs were taken and were measured wound space using Olympus DP controller software. The percentage of change in the wound space was calculated as follows:

$$\% \text{ Change} = \frac{(\text{average space at time 0 h}) - (\text{average space at time 24,48 h})}{(\text{average space at time 0 h})} \text{ X } 100$$

Invasion assay. An invasion assay was performed using a 24-well transwell unit with polycarbonate (PVDF) filters (8 mm pore size). Each membrane was coated with 0.5% matrigel (BD Biosciences, Bedford, MA, USA) on the upper surface of the chamber overnight at 37˚C in incubator. The cells were plated in upper chamber at the density of 2×10^4 cells/100 μl in serum-free medium and were incubated with cycloartobiloxanthone non- toxic concentrations (0-10 μM) medium containing 10% FBS was added to the lower chamber of the unit. After 24 h, the top medium and matrigel were removed and the cells on the upper side of the membrane were removed with a cotton swab. The bottom side was fixed with 3.7% paraformaldehyde and staining cells with Hoechst 33342. The cells were visualized and scored under a fluorescence microscope (Olympus IX51 with DP70, USA).

Cell morphology and filopodia characterization. Cell morphology and filopodia was investigated by a phalloidin-Hoechst 33342 staining assay. The cells were seeded in a 96-well plate at a density of 10^4 cells/well for 24 h. After treatment, the cells were washed with PBS and fixed with 4% paraformaldehyde in PBS for 10 min at 37˚C. Afterwards, cells were permeabilized with 0.1% Triton-x100 in PBS for 4 min and blocked with 0.2% bovine serum albumin (BSA) for 30 min. After blocking, cells were incubated with a phalloidin-Hoechst 33342 in PBS, and mounted with 50% glycerol. Cell morphology was assessed under a fluorescence microscope (Olympus IX51 with DP70). Filopodia protrusion was represented as the average number of filopodia per cell relative to untreated cells in each field.

Western Blot. The cells were seeded at a density of 3×10^5 cells onto 60 mm^2 plates overnight. After treatments, the cells were washed with cold PBS and incubated with a lysis buffer containing 20 mM Tris-HCl (pH 7.5), 150 mM sodium chloride (NaCl), 10% glycerol, 1 mM sodium orthovanadate, 50 mM sodium fluoride, 1 mM phenylmethylsulfonyl fluoride, and a protease inhibitor cocktail (Roche Molecular Biochemicals, Indianapolis, IN, USA) for 30 min on ice. Cell lysates were collected, and the protein content was determined by using the BCA protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA). The amount of protein from each

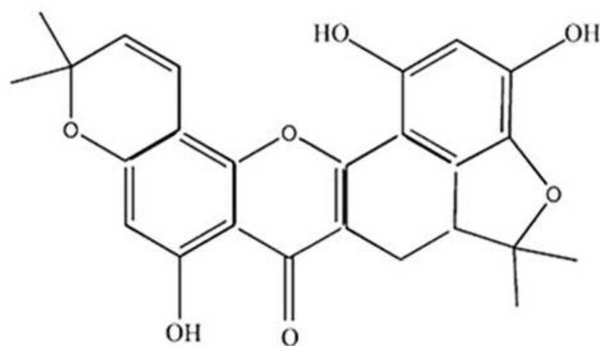

Figure 1. *Structure of cycloartobiloxanthone.*

sample (60 μg) was denatured by heating at 95˚C for 5 min and loaded onto 7.5-10% SDS-polyacrylamide gels. After separation, the proteins were transferred onto 0.45 μm nitrocellulose membranes (Bio-Rad), and the transferred membranes were blocked in 5% nonfat dry milk in TBST (25 mM Tris-HCl (pH 7.5), 125 mM NaCl, 0.05% Tween 20) for 1 h and subsequently incubated with a specific 1˚ antibody overnight at 4˚C. Then, the membranes were washed three times in 5 mins with TBST and incubated with horseradish peroxidase (HRP) – conjugated anti-rabbit or anti-mouse IgG isotype-specific secondary antibodies in 5% nonfat dry milk for 2 h at room temperature. After three washes with TBST, the immune complexes were detected using chemiluminescence (Supersignal West Pico; Pierce, Rockford, IL, USA) and quantified using the analyst/PC densitometry software (Bio-Rad).

Statistical analysis. Mean data from at least three independent experiments was normalized to the results of the control group. Statistical differences between two groups was determined by Student's *t*-test, and the use of an analysis of variance (ANOVA) with a *post-hoc* test to compare the multiple groups at a significance level of $p<0.05$. The data is presented as the mean±SD.

Results

Cytotoxic effect of cycloartobiloxanthone on human non-small cell lung cancer H460 cells. The previous study (14) suggests that cycloartobiloxanthone (Figure 1) has DPPH free radical scavenging activity and inhibitory effect on production of nitric oxide in murine macrophage-like cells (14). However, scientific evidence regarding the anti-metastasis activity of cycloartobiloxanthone is not known and could be beneficial for the development of this compound for further clinical study.

First, we characterized the cytotoxicity of cycloartobiloxanthone on human lung cancer H460 cells. Briefly, cells were incubated in culturing medium in the presence or absence of cycloartobiloxanthone (0-100 μM) for 24 h and cell viability was analyzed by the MTT assay. Figure 2A shows that cycloartobiloxanthone at the concentrations of more than 20 μM causes a significant decrease in viability of cancer cells, while the concentrations of less than 10 μM showed no significant effect. Data analysis indicated that the IC_{50} of cycloartobiloxanthone was approximately 61.82 μM. Furthermore, the apoptosis and necrosis of the cells were evaluated by Hoechst33342 and propidium iodide nuclear staining. The nuclear morphology study of control and cycloartobiloxanthone-treated cells shown in Figure 2B and C supported the above findings that apoptotic cell death was detected in response to cycloartobiloxanthone at indicated concentrations.

Cycloartobiloxanthone inhibits migration and invasion of H460 cells. This study aimed to investigate the anti-migration and anti-invasion activities of cycloartobiloxanthone, the non-toxic concentrations (0-10 μM) of the compound were then used to treat cells. To examine the effect of cycloartobiloxanthone on migration of the cells, a wound-healing assay was performed. The confluent monolayer of H460 cells was scratched and cells were cultured with or without non-toxic concentrations of cycloartobiloxanthone (0-10 μM) for 24 and 48 h. Figures 3A and B show that the incubation of cells with cycloarto-biloxanthone at a concentration of 5 and 10 μM significantly reduced the motility of H460 cells to the wound area, whereas cycloartobiloxanthone at 1 μM had no significant effect on cell migration in comparison to that of control at 24 and 48 h. These results indicate that cycloartobiloxanthone possesses the ability to inhibit cancer cell migration.

For invasion assay, H460 cells were added to a 24-well transwell pre-coated with Matrigel and then treated with non-toxic concentrations of cycloartobiloxanthone. As shown in Figure 3C and D, cycloartobiloxanthone at all treated concentrations significantly inhibited cancer cell invasion through the Matrigel matrix.

Cycloartobiloxanthone inhibits filopodia formation in lung cancer cells. During cell movement, the formation of filopodia (plasma membrane protrusions) tends to increase and it is involved in cancer cell migration (19). This study further tested the effect of cycloartobiloxanthone on filopodia formation. H460 cells were treated with 0-10 μM cycloartobiloxanthone and phalloidin-labeled filopodia were detected under fluorescence microscopy. The results show that in untreated controls, cells showed several membrane protrusions of filopodia (Figure 4A and B). Remarkably, treatment with cycloartobiloxanthone significantly suppressed filopodia formation in H460 cells when compared with untreated cells. This data and the above findings suggest that cycloartobiloxanthone not only has potential to decrease cell migration and invasion but also reduce cellular filopodia.

Cycloartobiloxanthone inhibits FAK, CDC42 signaling and suppresses integrin expression in H460 cells. To understand the mechanism of cycloartobiloxanthone in suppression of

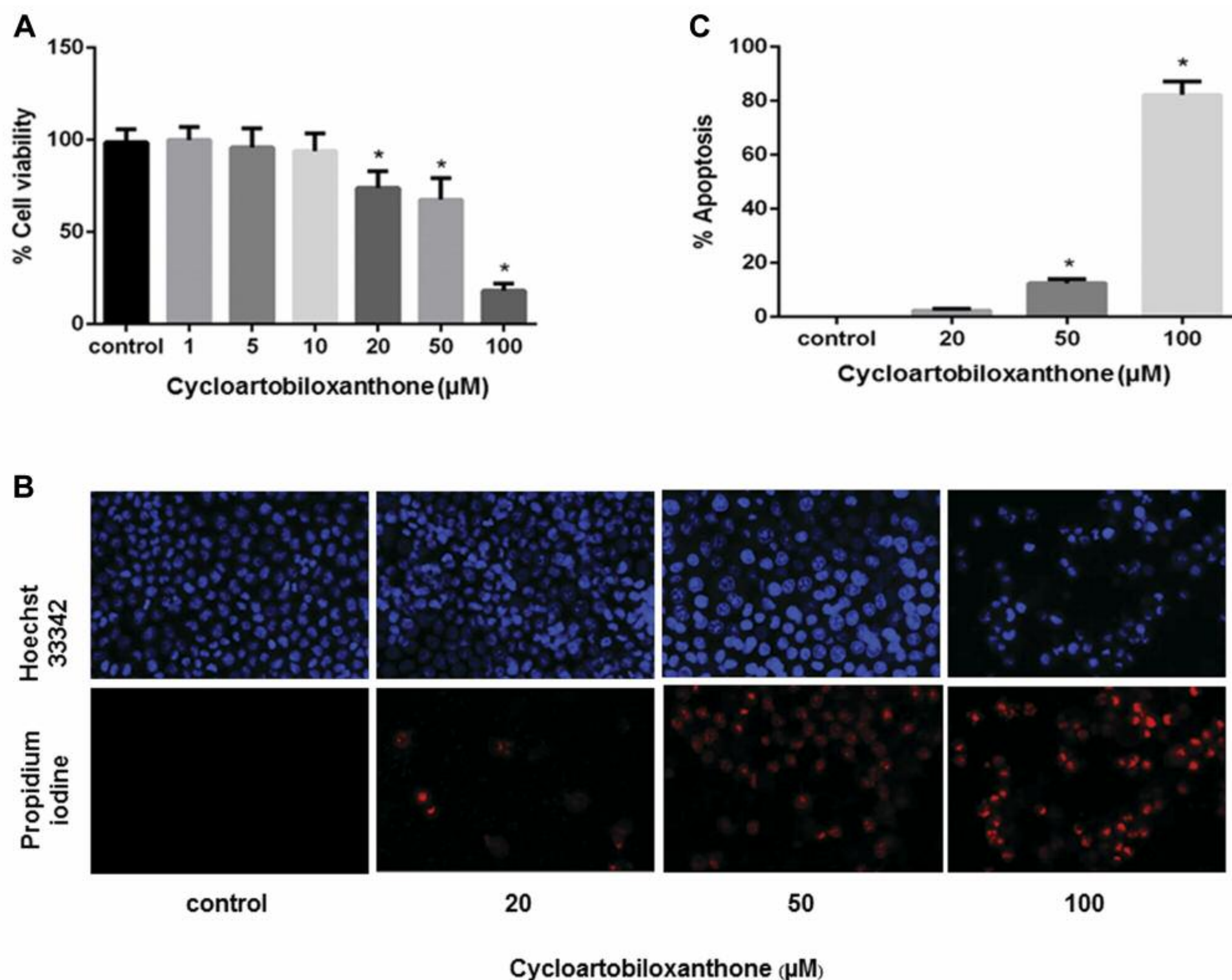

Figure 2. *Effect of cycloartobiloxanthone on cell viability of human lung cancer H460 cells. The cells were treated with 0-100 μM of cycloartobiloxanthone for 24 h. A: Percentage of cell viability and cell apoptosis were analyzed by MTT assay and Hoechst 33342/propidium iodine staining assays, respectively. B: Morphology of apoptotic nuclei stained with Hoechst 33342 and propidium iodide (PI). C: Percentage of apoptosis cells were analyzed by staining with Hoechst 33342 and propidium iodide. Values are means of triplicate samples±SD.*

cancer cell motility, the expression level and activation status of proteins regulating cell motility such as FAK and AKT, were investigated. 3×10^5 cells were seeded and incubated with 0-10 μM cycloartobiloxanthone and the expression of proteins was determined by western blotting. Cycloartobiloxanthone significantly reduced on the level of active FAK indicated by the reduction of p-FAK/FAK ratio at the concentrations of 5-10 μM. The phosphorylation of FAK at Tyr 397 activates its kinase activity that triggers AKT signaling (17). We, thus, investigated the possible effect of cycloartobiloxanthone on AKT activation. Figure 5C demonstrates that cycloartobiloxanthone had no significant effect on phosphorylation of AKT at Ser 473 and AKT expression in a concentration-dependent manner. These findings suggested that cycloartobiloxanthone inhibits cell migration *via* FAK dependent mechanism but independent of the down-regulation of AKT and AKT at Ser 473.

Interestingly, evidence suggests that the increasing of filopodia at the boundary of moving cells is regulated by Cdc42 (18). To test whether the decrease in the number of filopodia protrusions involved Cdc42, cells were treated with non-toxic concentrations for 24 h and determined by western blotting. The result shows that Cdc42 was substantially down-regulated when compared with untreated cells (Figure 5C). These findings suggested that cycloartobiloxanthone reduces the migratory behavior of lung cancer cells *via* Cdc42 attenuation and filopodia suppression.

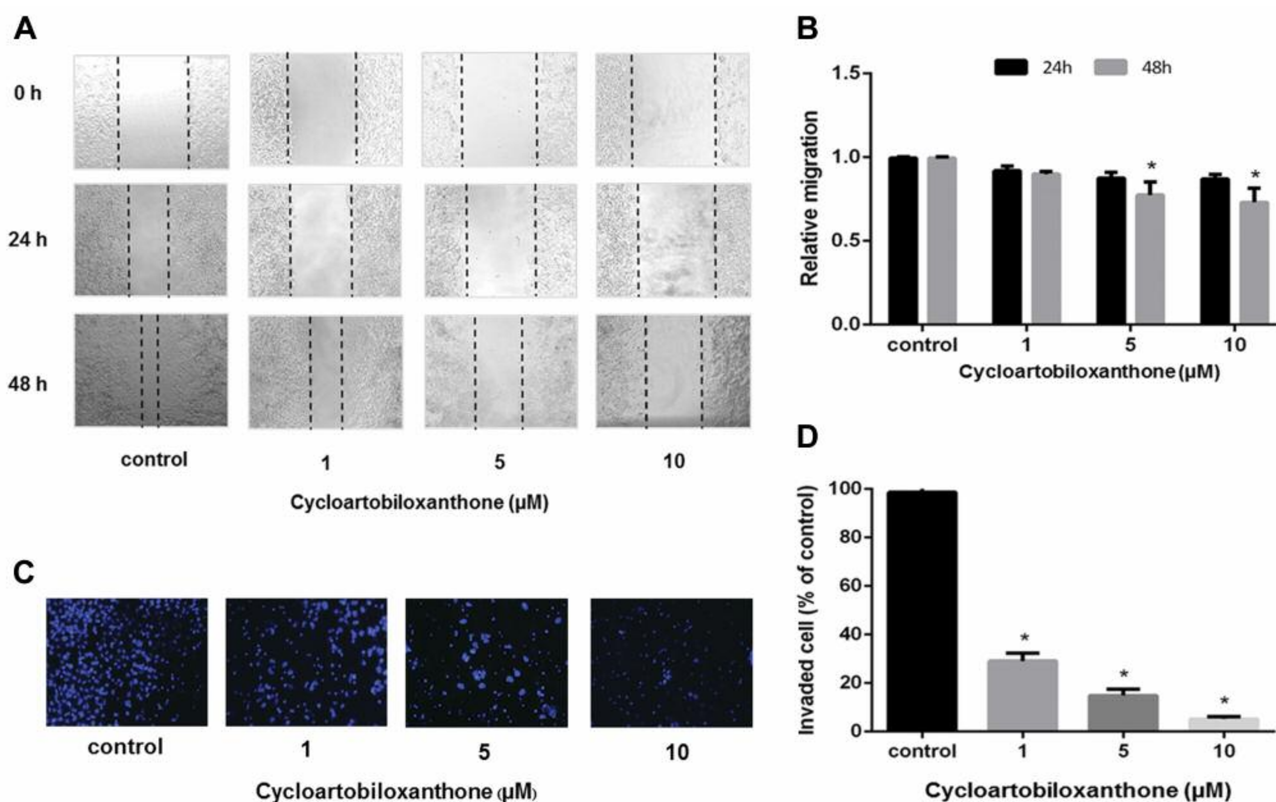

Figure 3. *The effects of cycloartobiloxanthone on H460 cell migration and invasion. For migration assay, a wound-healing assay was performed. Wound space was created by 1-mm tips and the cells were treated with sub-toxic concentrations of cycloartobiloxanthone for 24 and 48 h. For invasion assays, the cells were seeded onto Matrigel coated membrane and treated with sub-toxic concentrations of cycloartobiloxanthone for 24 h. A: Wound space was pictured under a phase-contrast microscope at the 0, 24 and 48 h. B: The relative cell migration was analyzed by comparison of the relative change in wound space of the control groups over that of the untreated control. C, D: The invaded cells were stained with Hoechst 33342, pictured under a fluorescence microscopy, and the relative cell invasion was determined. The values are means of triplicate samples±SD.; *p<0.05 versus untreated control.*

Integrins are essential for cell invasion and migration in cancer cells. Integrins activate kinases that phosphorylate cytoskeletal proteins, regulating stress-fiber formation, cell shape and migration (19). To study integrin proteins involved in the ability of cycloartobiloxanthone to inhibit cell motility, H460 cells were incubated with various concentrations of the compound (0-10 μM) for 24 h and migratory relating proteins were examined by Western blot analysis. Figure 5A shows that while cycloartobiloxanthone had only a minimal effect on the level of total integrin β1, the treatment of cycloartobiloxanthone at concentrations of 5-10 μM significantly reduced the level of β3 and αV. In addition, the level of α5 significantly reduced at all concentrations (1-10 μM). These results suggest that cycloartobiloxanthone attenuates lung cancer cell migration and invasion through suppress levels of integrin expression in H460 cells.

Cycloartobiloxanthone transforms cell from mesenchymal to epithelium cell and up-regulates E-cadherin in H460 cells. E-cadherin is a transmembrane protein that mediates homophilic cell–cell interactions. E-cadherin actions as a tumor suppressor inhibiting invasion and metastasis. Both epithelial–mesenchymal transition (EMT) and mesenchymal–epithelial transition (MET) are dependent on E-cadherin, and cells undergoing MET will up-regulate E-cadherin resulted in change morphology such as organized interactions among cells and facilitating the formation of continuous cell layers (20). To investigate cell morphology, cells were stained with phalloidin and Hoechst33342 in different non-toxic concentrations. Figure 6A shows that at 5 and 10 μM of cycloartobiloxanthone cell were form continuous cell layers and presence of tight junctions.

Furthermore, tumor progression is often associated with the loss protein level of E-cadherin resulted in more motile and

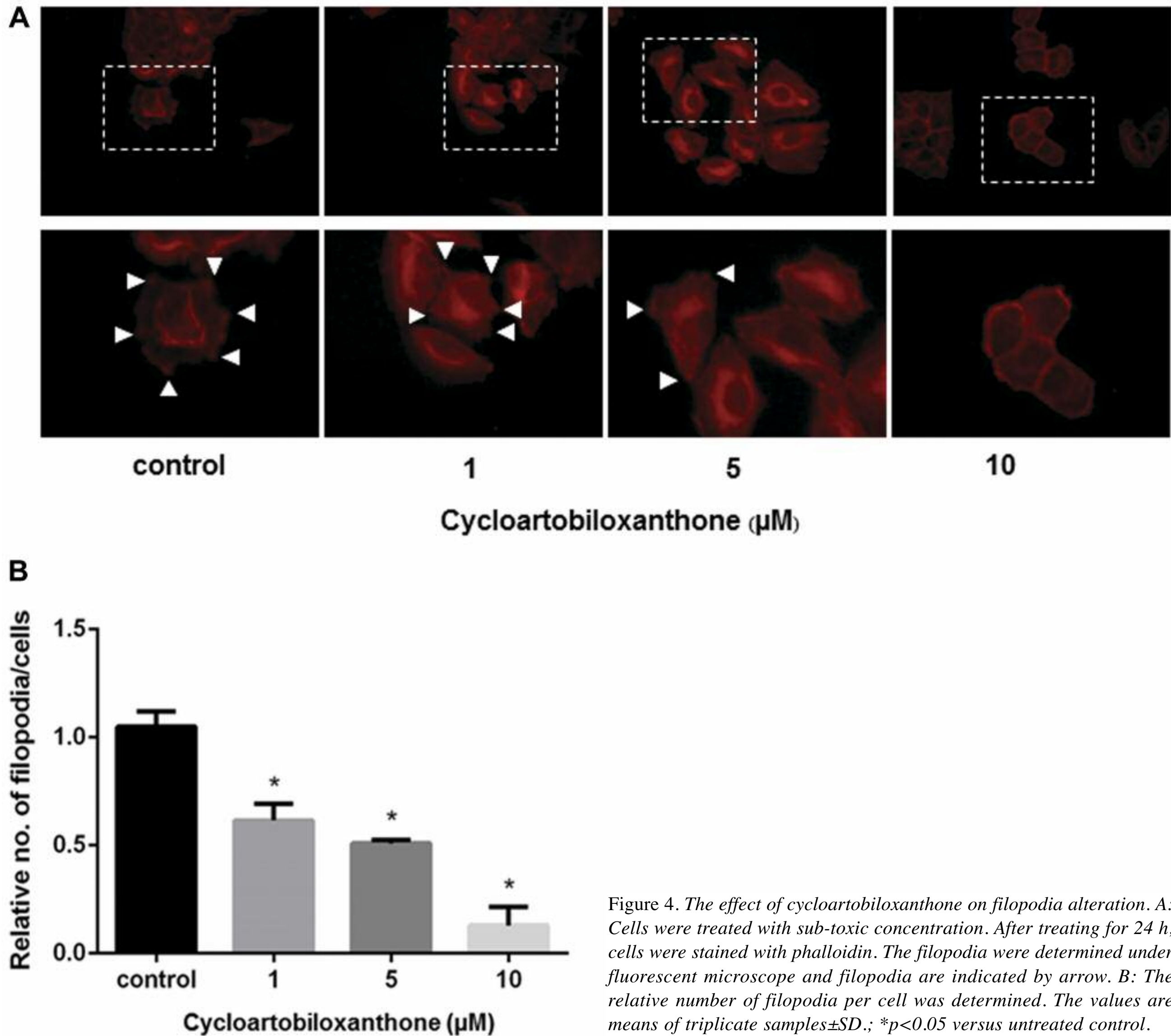

Figure 4. *The effect of cycloartobiloxanthone on filopodia alteration. A: Cells were treated with sub-toxic concentration. After treating for 24 h, cells were stained with phalloidin. The filopodia were determined under fluorescent microscope and filopodia are indicated by arrow. B: The relative number of filopodia per cell was determined. The values are means of triplicate samples±SD.; *$p<0.05$ versus untreated control.*

invasive (21). To investigate the effect of cycloartobiloxanthone on E-cadherin in H460 cells, cells were treated with non-toxic concentrations and then evaluate by western blot analysis. Our results, shown in Figure 6B and C, revealed that cyclo-artobiloxanthone at 5 and 10 μM significantly increased E-cadherin in comparison to untreated controls. These data and the above findings suggest that cycloartobiloxanthone has a positive impact on cell morphology, involve up-regulation of E-cadherin.

Discussion

In order for cancer cells to metastasize and grow, cells must invade and migrate into nearby tissues. Cancer metastasis is the primary cause of death in cancer patients, especially in lung cancer patients (19). Herein, we demonstrate that cycloartobiloxanthone plays a role in inhibiting lung cancer cell migration and invasion. Previous studies shown that nitric oxide has ability to induce invasion and metastasis (22). Cycloartobiloxanthone has inhibitory effect on nitric oxide (14), therefore, cycloartobiloxanthone exhibited the inhibitory effect on cell invasion and migration in lung cancer. The migration of cancer cells involves multiple mechanistic pathways and most of them involve the invasive function including FAK (23), AKT (24) and Cdc42 (25). Cell migration and invasion can be stimulated by FAK signaling pathways. Similarly, previous studies exhibit that phosphorylation of FAK at Tyr 397 is necessary for FAK to elevate in highly motile and invasive cancer cells (26). Likewise, phosphorylation of PI3K

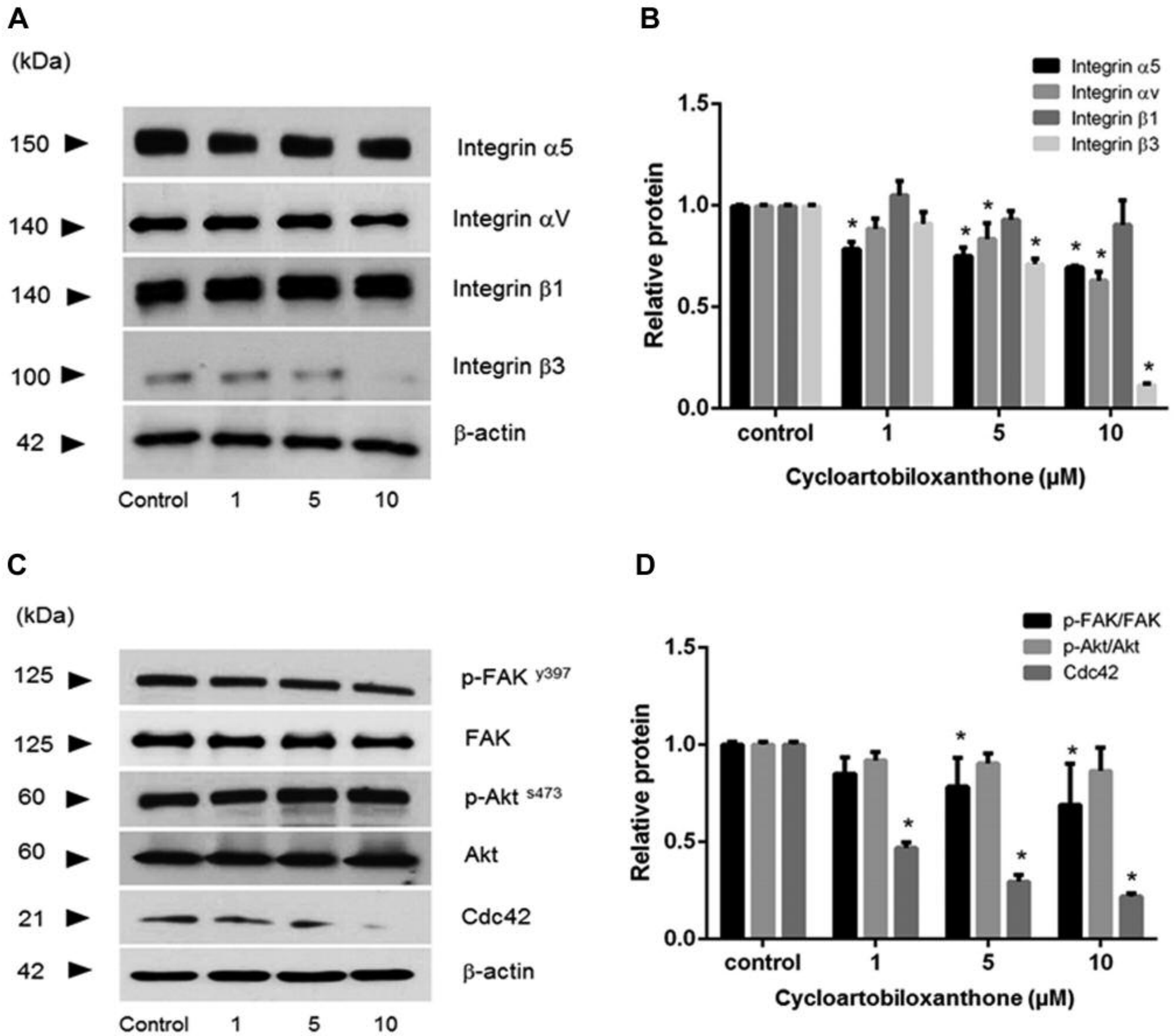

Figure 5. *The effect of cycloartobiloxanthone on intergrins, focal adhesion kinase (FAK), protein kinase B (AKT) and cell division cycle (Cdc42) proteins. A: The cells were treated with 0-10 μM cycloartobiloxanthone for 24 h. The expressions of integrin αV, α5, β1 and β3 proteins were determined by western blotting. B: The relative proteins were quantified by densitometry and mean data from three independent experiments were presented. Values are means of samples±SD. (n=3); *p<0.05 versus untreated group. C: The cells were seeded and treated with 0-10 μM of cycloartobiloxanthone for 24 h. The expression of pFAK (Tyr 397), FAK, pAKT (Ser 473), AKT and Cdc42 proteins were determined by western blotting. D: The immunoblot signals were quantified by densitometry and mean data from three independent experiments were presented. Values are means of samples±SD, (n=3); *p<0.05 versus untreated group.*

by FAK may activate PI3K/Akt signaling pathway that was shown to be associated with cell migration and invasion (27). For instance, FAK has been shown to be capable of regulating integrin-mediated signaling due to FAK is primarily recruited to sites of integrin clustering *via* interactions between its C-terminal domain and integrin-associated proteins such as talin and paxillin (28). Recent data has suggested that integrins, a class of cell surface adhesive receptors that are found as a αβ heterodimers, mediate many of cellular behaviors, especially cell motility. In cancer cell biology, the alteration of these proteins or integrins switch was shown to be important metastasis (29). Integrin switch not only promotes NSCLC migration and invasion but also promotes chemotherapeutic drug resistance (30). The integrin expression pattern is

Figure 6. *The effect of cycloartobiloxanthone on mesenchymal and epithelium morphology and the expression of epithelium protein of human lung cancer H460 cells. The cells were treated with 0-10 μM of cycloartobiloxanthone for 24 h. A: Morphology of mesenchymal and epithelium cells stained with Hoechst 33342 and phalloidin. B: The cells were seeded and treated with 0-10 μM of cycloartobiloxanthone for 24 h. The expressions of E-cadherin were determined by western blotting. C: The relative proteins were quantified by densitometry and mean data from three independent experiments were presented. Values are means of samples±SD. (n=3); *p<0.05 versus untreated group.*

regulated by cellular mechanisms responding to the extracellular and microenvironmental signals. For instance, we have provided evidence indicating that the exposure to sub toxic concentration of anti-cancer drug cisplatin conferred up-regulation of integrin α4, αV, β1 and β5 and increased lung cancer cell motility (31). In particular, certain integrins have been shown to play roles in enhancing cancer metastasis. The suppression of such integrins could suppress cancer aggressive behaviors. Previously, the down-regulation of β3-integrin mediated by siRNA was shown to dramatically inhibited bone metastasis of lung cancer (32).

Cdc42, this is a protein that in Rho GTPase family, is also associated in filopodia formation which leads to migration and invasion of cancer cells (33). Also, declined Cdc42 expression has been informed to inhibit cell migration (34). In the present study, we found that treatment of lung cancer H460 cells with cycloartobiloxanthone resulted in the reduction of the cellular Cdc42 levels (Figure 5C and D), along with the findings indicating that filopodia protrusions were reduced in the cells treated with cycloartobiloxanthone (Figure 4A and B). This compound may, at least in part, inhibit migration and invasion through Cdc42 suppression.

In addition, we provided evidence indicating that the inhibitory effect of cycloartobiloxanthone on cancer cell motility can also be attributed to up-regulated E-cadherin (Figure 6B and C) which is a key mediator of cell–cell adhesions in epithelial tissues, and loss of E-cadherin can promote invasive and metastatic behavior in many epithelial tumors (35).

In summary, this investigation reveals that cycloartobiloxanthone can inhibit migration and invasion of lung cancer cells *via* suppression of activated FAK, Cdc42 and integrins; therefore, antimigratory activity of cycloartobiloxanthone could provide the basis for the future development of this compound for antimetastasis therapy or used as adjuvant with standard therapies to improve survival rate of patients with cancer.

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Conflicts of Interest

The Authors declare that there is no conflict of interest in regard to this research.

References

1 Siegel RL, Miller KD and Jemal A: Cancer statistics, 2016. CA: a cancer journal for clinicians *66*: 7-30, 2016.

2 Travis WD, Travis LB and Devesa SS: Lung cancer. Cancer *75*: 191-202, 1995.

3 Shieh DB, Godleski J, Herndon JE, 2nd, Azuma T, Mercer H, Sugarbaker DJ and Kwiatkowski DJ: Cell motility as a prognostic factor in Stage I nonsmall cell lung carcinoma: the role of gelsolin expression. Cancer *85*: 47-57, 1999.

4 Nagamachi Y, Tani M, Shimizu K, Tsuda H, Niitsu Y and Yokota J: Orthotopic growth and metastasis of human non-small cell lung carcinoma cell injected into the pleural cavity of nude mice. Cancer letters *127*: 203-209, 1998.

5 Keshamouni V, Arenberg D and Kalemkerian G: Lung cancer metastasis: Novel biological mechanisms and impact on clinical practice. Springer-Verlag, New York, NY, USA, 2009.

6 Mina LA and Sledge GW: Rethinking the metastatic cascade as a therapeutic target. Nat Rev Clin Oncol *8(6)*: 325-332, 2011.

7 Pongrakhananon V, Chunhacha P and Chanvorachote P: Ouabain suppresses the migratory behavior of lung cancer cells. PloS one *8*: e68623, 2013.

8 Harlozinska A: Progress in molecular mechanisms of tumor metastasis and angiogenesis. Anticancer Res *25(5)*: 3327-3333, 2005.

9 Mitra SK and Schlaepfer DD: Integrin-regulated FAK–Src signaling in normal and cancer cells. Current Opinion in Cell Biology *18*: 516-523, 2006.

10 Bolos V, Gasent JM, Lopez-Tarruella S and Grande E: The dual kinase complex FAK-SRC as a promising therapeutic target in cancer. Onco Targets Ther *3*: 83-97, 2010.

11 Chen QY, Jiao DM, Yao QH, Yan J, Song J, Chen FY, Lu GH and Zhou JY: Expression analysis of Cdc42 in lung cancer and modulation of its expression by curcumin in lung cancer cell lines. International journal of oncology *40*: 1561-1568, 2012.

12 Morel AP, Lièvre M, Thomas C, Hinkal G, Ansieau S and Puisieux A: Generation of breast cancer stem cells through epithelial-mesenchymal transition. PLoS one *3(8)*: e2888, 2008.

13 Thiery JP, Acloque H, Huang RY and Nieto MA: Epithelial-mesenchymal transitions in development and disease. Cell *139*: 871-890, 2009.

14 Sritularak B, Tantituvanont A, Chanvorachote P, Meksawan K, Miyamoto T, Kohno Y and Likhitwitayawuid K: Flavonoids with free radical-scavenging activity and nitric oxide inhibitory effect from the stem bark of *Artocarpus gomezianus*. J Med Plants Res *4(5)*: 387-392, 2010.

15 Baramee A, Kittakoop P and Puangsombat P: Antitubercular and Antiplasmodial Prenylated Flavones from the Roots of *Artocarpus altilis*. Chiang Mai J Sci *34(3)*: 339-344, 2007.

16 Machesky LM: Lamellipodia and filopodia in metastasis and invasion. FEBS letters *582*: 2102-2111, 2008.

17 Bolós V, Gasent JM, López-Tarruella S and Grande E: The dual kinase complex FAK-Src as a promising therapeutic target in cancer. OncoTargets and therapy *3*: 83-97, 2010.

18 Arjonen A, Kaukonen R and Ivaska J: Filopodia and adhesion in cancer cell motility. Cell adhesion & migration *5*: 421-430, 2011.

19 Hood JD and Cheresh DA: Role of integrins in cell invasion and migration. Nature reviews Cancer *2*: 91-100, 2002.

20 Canel M, Serrels A, Frame MC and Brunton VG: E-cadherin-integrin crosstalk in cancer invasion and metastasis. Journal of cell science *126*: 393-401, 2013.

21 Larue L and Bellacosa A: Epithelial-mesenchymal transition in development and cancer: role of phosphatidylinositol 3' kinase/AKT pathways. Oncogene *24*: 7443-7454, 2005.

22 Saisongkorh V, Maiuthed A and Chanvorachote P: Nitric oxide increases the migratory activity of non-small cell lung cancer cells *via* AKT-mediated integrin alphav and beta1 up-regulation. Cellular oncology (Dordrecht) *39*: 449-462, 2016.

23 Bolos V, Gasent JM, Lopez-Tarruella S and Grande E: The dual kinase complex FAK-SRC as a promising therapeutic target in cancer. Onco Targets Ther *3*: 83-97, 2010.

24 Kim D, Kim S, Koh H, Yoon SO, Chung AS, Cho KS and Chung J: Akt/PKB promotes cancer cell invasion *via* increased motility and metalloproteinase production. FASEB J *15(11)*: 1953-1962, 2001.

25 Sinha S and Yang W: Cellular signaling for activation of Rho GTPase CDC42. Cell Signal *20(11)*: 1927-1934, 2008.

26 Zhao J and Guan JL: Signal transduction by focal adhesion kinase in cancer. Cancer Metastasis Rev *28*: 35-49, 2009.

27 Grille SJ, Bellacosa A, Upson J, Klein-Szanto AJ, van Roy F, Lee-Kwon W, Donowitz M, Tsichlis PN and Larue L: The protein kinase Akt induces epithelial- mesenchymal transition and promotes enhanced motility and invasiveness of squamous cell carcinoma lines. Cancer Res *63(9)*: 2172-2178, 2003.

28 Mitra SK and Schlaepfer DD: Integrin-regulated FAK-Src signaling in normal and cancer cells. Curr Opin Cell Biol *18*: 516-523, 2006.

29 Desgrosellier JS and Cheresh DA: Integrins in cancer: biological implications and therapeutic opportunities. Nat Rev Cancer *10*: 9-22, 2010.

30 Aoudjit F and Vuori K: Integrin signaling in cancer cell survival and chemoresistance. Chemotherapy research and practice *2012*: 283181, 2012.

31 Maiuthed A and Chanvorachote P: Cisplatin at sub-toxic levels mediates integrin switch in lung cancer cells. Anticancer research *34*: 7111-7117, 2014.

32 Li N, Zhang J-p, Guo S, Min J, Liu Ll, Su H-c, Feng Y-m and Zhang H-l: Down-regulation of β3-integrin inhibits bone metastasis of small cell lung cancer. Molecular Biology Reports *39*: 3029-3035, 2012.

33 Machesky LM: Lamellipodia and filopodia in metastasis and invasion. FEBS Lett *582(14)*: 2102-2111, 2008.

34 Cheng WY, Chiao MT, Liang YJ, Yang YC, Shen CC and Yang CY: Luteolin inhibits migration of human glioblastoma U-87 MG and T98G cells through down-regulation of Cdc42 expression and PI3K/AKT activity. Mol Biol Rep *40*: 5315-5326, 2013.

35 Birchmeier W and Behrens J: Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. Biochimica et biophysica acta *1198*: 11-26, 1994.

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