Nutrition and Cancer

ISSN: 0163-5581 (Print) 1532-7914 (Online) Journal homepage: https://www.tandfonline.com/loi/hnuc20

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To cite this article: Wipa Chantarawong, Nattakorn Kuncharoen, Somboon Tanasupawat & Pithi Chanvorachote (2019): Lumichrome Inhibits Human Lung Cancer Cell Growth and Induces Apoptosis via a p53-Dependent Mechanism, Nutrition and Cancer, DOI: 10.1080/01635581.2019.1610183

To link to this article: https://doi.org/10.1080/01635581.2019.1610183

NUTRITION AND CANCER
https://doi.org/10.1080/01635581.2019.1610183

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Lumichrome Inhibits Human Lung Cancer Cell Growth and Induces Apoptosis via a p53-Dependent Mechanism

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ABSTRACT

Lumichrome, a major derivative of riboflavin, may exhibit pharmacological activity against cancer cells. Riboflavin is a vitamin found in food, however, certain evidence has suggested its possible potentiating effects on cancer progression. Here, we have shown for the first time that unlike riboflavin, lumichrome can suppress lung cancer cell growth and reduce survival in both normal and anchorage-independent conditions. In addition, lumichrome induced apoptosis in lung cancer cells via a p53-dependent mitochondrial mechanism with substantial selectivity, shown by its lesser toxicity to the normal primary dermal papilla cells. The potency of lumichrome in killing lung cancer cells was found to be comparable to that of cisplatin, a standard chemotherapeutic drug for lung cancer treatment. With regard to the mechanism, lumichrome significantly upregulated p53 and decreased its downstream target BCL-2. Such a shift of BCL-2 family protein balance further activated caspase-9 and -3 and finally executed apoptosis. Furthermore, lumichrome potentially suppressed cancer stem cells (CSCs) in lung cancer by dramatically suppressing CSC markers together with the CSC-maintaining cell signaling namely protein kinase B (AKT) and β-catenin. To conclude, the present study has unraveled a novel role and mechanism of lumichrome against lung cancer that may benefit the development of the compound for management of the disease.

ARTICLE HISTORY
Received 14 December 2018
Accepted 16 April 2019

Introduction

Lung cancer is one of the most frequently diagnosed cancers and causes the largest number of cancer-related deaths in the world (1). There were approximately two million newly diagnosed cases of lung cancer in 2018 (1). Usually lung cancer exhibits a prompt local and systemic metastasis with high resistance to conventional antineoplastic therapies (2). Moreover, most currently used therapeutic agents usually cause serious toxicity due to the lack of cancer-cell specificity (3). Thus, development of new therapeutic agents with safe profile is urgently required to improve the clinical outcomes for cancer therapy. One principle of cancer therapy is to eliminate cancer cells via an apoptosis mechanism. Chemotherapeutic agents can induce apoptosis in cancer cells via induction of a DNA-damage signal or DNA adducts which trigger the activation of the p53 protein (4). p53 has the ability to induce transcription of pro-apoptotic genes, including B-cell lymphoma 2 (BCL2)-associated X (BAX) (5,6). In addition, p53 reduces the expression of anti-apoptotic proteins, such as BCL2 (5,6). The induction of pro-apoptotic protein expression subsequently causes mitochondrial outer membrane permeabilization (MOMP), the release of cytochrome c, caspase activation and poly (ADP-ribose) polymerase (PARP) cleavage, leading to apoptosis (7).

Recent research has advocated the presence of a rare population of cancer stem cells (CSCs) which are responsible for cancer initiation, drug resistance, metastasis, and relapse (8,9). These CSCs are also known as cancer-initiating cells (CICs) (10). CSCs have certain characteristics, which is the capacity to self-renew and to cause the heterogeneous lineages comprising tumors (11). As these subpopulations are believed to be a critical cause of aggressive behaviors of lung cancers, many attempts have been made to discover new compounds targeting CSCs (12). The augmented and sustained levels of survival signals have been demonstrated as one of the hallmarks of cancer (13). Protein kinase B (AKT) is a central

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survival signal that has been linked with poor prognosis in various cancers including lung cancer (14–16). Furthermore, AKT has been shown to be related to chemotherapeutic drug resistance, especially in platinum-based chemotherapy for lung cancer (17,18). Interestingly, the AKT pathway is essential for CSC maintenance, either by activating mTOR signals (19) or increasing the cellular level and function of β-catenin (20,21). In detail, AKT can phosphorylate β-catenin at Ser552. Such a phosphorylation increases β-catenin stability and confers its transcriptional activity, thereby increasing cellular stem cell signals (22,23). In supporting, available evidence indicates that β-catenin signaling considerably influences lung cancer carcinogenesis, progression, and resistance to therapy, implying that β-catenin pathway antagonists may benefit the treatment of lung cancer (24). Likewise, active AKT activated the function of mTOR and STAT3, which has been shown to be important for CSCs (19,25).

Lumichrome (7,8-dimethylalloxazine) is one major derivative of vitamin B2 or riboflavin (7,8-dimethyl-10-ribityl-isoalloxazine). Riboflavin plays an important role in protein, fat, and carbohydrate metabolism and is critical for maintaining energy supply (26). Regarding the effect of riboflavin on cancer, one piece of evidence has suggested that high doses of riboflavin might promote lung cancer progression (27). In addition, riboflavin deficiency has inhibited tumor growth in experimental animals (28). Unlike riboflavin, only limited information has been reported on the effect of lumichrome on cancer cells.

In this study, we demonstrate that lumichrome extracted from the culture broth of *Streptomyces pseudovenezuelae* SKH1-2 suppressed growth, mediated apoptosis, and suppressed CSCs in human non-small lung cancer cells. These findings might benefit the development of this relatively safe compound for possible anti-cancer approaches.

Materials and Methods

Reagents and Antibodies

Roswell Park Memorial Institute (RPMI) 1640 medium, Dulbecco's Modified Eagle's Medium (DMEM) medium, fetal bovine serum (FBS), L-glutamine, penicillin/streptomycin, phosphate-buffered saline (PBS), and trypsin-EDTA were obtained from GIBCO (Grand Island, NY, USA). Riboflavin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT), dimethyl sulfoxide (DMSO), Hoechst33342, propidium iodide (PI), cisplatin and bovine serum albumin (BSA) were obtained from Sigma-Aldrich, Co. (St. Louis, MO, USA). Primary antibodies to PARP, caspase-9, caspase-3, p-53, BCL-2, BAX, AKT, CD44, CD133, β-catenin and β-actin, and the respective secondary antibodies, were obtained from Cell Signaling (Danvers, MA, USA).

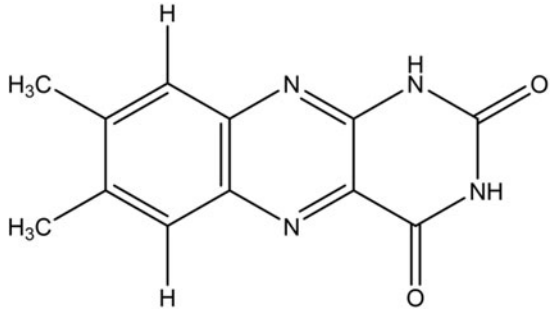


Figure 1. Chemical structure of lumichrome.

Lumichrome Preparation

Lumichrome ($C_{12}H_{10}N_4O_2$), a yellow solid compound, extracted and purified from the culture broth of *Streptomyces pseudovenezuelae* SKH1-2 was obtained from Nattakorn Kuncharoen and Somboon Tanasupawat, Department of Biochemistry and Microbiology, the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand. The structure of the compound is given in the Figure 1. Lumichrome was dissolved in DMSO at the indicated working concentrations. The amount of DMSO in the final concentration was less than 0.1%, which showed no toxicity in cancer and normal cells.

Cell Lines and Culture

Human non-small cell lung cancer (NSCLC) cell lines, H292, H460, and A549 cells and human colorectal carcinoma (HCT116) cells were obtained from the American Type Culture Collection (Manassas, VA, USA). Human dermal papilla primary (Primary DP1) cells were obtained from Celprogen (Benelux, Netherland). Human primary hair follicle dermal papilla (Primary DP2) cells and Immortalized dermal papilla (DP) cells were obtained from Applied Biological Materials Inc (Richmond, BC). H292, H460, and HCT116 cells were cultured in RPMI 1640 medium. A549, DP, Primary DP1, and Primary DP2 were cultured in DMEM medium. The medium was supplemented with 10% FBS, 2 mM L-glutamine and 100 units/ml of each penicillin and streptomycin at 37 °C with 5% CO2 in a humidified incubator.

Cell Viability Assay

MTT assay was used to determine cell viability inhibition effect of lumichrome on cancer and normal cells. In brief, cells (1×10^4 cells/well) were seeded in 96-well plates, after overnight incubation, cells were incubated with different concentrations of lumichrome (0, 5, 10, 25, 50, and 100 µM) for 24 h. After that, 100 μl of MTT solution (400 µg/ml) was added, incubated for another 4 h at 37 °C, and then 100 μl of DMSO was added to dissolve the formazan crystal product. Absorbance was measured by spectrophotometry at 570 nm using a microplate reader (Anthros, Durham, NC, USA).

Colony Formation Assay

H292, H460, and A549 cells pretreated with lumichrome (0, 10, 25, and 50 μM) for 24 h were seeded in 24-well plates at a density of 100 cells/well and incubated for 7 days. The colony was fixed with methanol at 4 °C for 15 min and stained with 0.1% crystal violet for 15 min. Colony formation was assessed using a phase-contrast microscope (Olympus IX5) equipped with a DP70 digital camera system (Olympus, Tokyo, Japan).

Anchorage-Independent Growth Assay

Anchorage-independent growth was examined by the soft agar colony-formation assay. H292, H460, and A549 cells were pretreated with lumichrome (0, 10, 25, and 50 μM) for 24 h. Soft agar was prepared by using a 1:1 mixture of medium containing 10% FBS and 1% agarose, and then 500 μl of the mixture was added in a 24-well plate and allowed to solidify at 4 °C for 15 min. To prepare the upper layer, cells (3×10^3 cells/ml) were mixed with medium containing 10% FBS and 0.3% agarose and then added over the bottom layer. After the upper layer solidified, the cultured medium was added and incubated at 37 °C for two weeks. Fresh medium (200 μl/well) was added every three days. Colony formation was assessed using a phase-contrast microscope (Olympus IX5).

Nuclear Staining Assay

H292, H460, and A549 cells were seeded in 96-well plates (1×10^4 cells/well) and treated with different concentrations of lumichrome (0–100 μM) and riboflavin for 24 h. After that, the cells were stained with 10 μg/ml Hoechst 33342 and 5 μg/ml PI for 15 min at 37 °C. Apoptotic cells exhibited nuclear condensation and DNA fragmentation stained by Hoechst 33342, while necrotic cells were characterized by PI staining. PI cannot pass through intact cell membranes, unless there is any damage leading to change in permeability of cell membrane. Nuclear condensation and DNA fragmentation of apoptotic cells and PI-positive necrotic cells were determined using a fluorescence microscope (Olympus IX5).

Western Blot Analysis

After lumichrome treatment, H292, H460, and A549 cells were incubated with RIPA lysis buffer supplemented with protease inhibitor cocktail (Roche Molecular Biochemical) for 30 min on ice. The extracted proteins (50 μg) were separated by SDS-PAGE and transferred to 0.45 μm nitrocellulose membranes (Bio-Rad). The membranes were blocked with 5% nonfat dry milk in TBST (Tris-buffer saline with 0.1% Tween containing 25 mM Tris-HCl (pH 7.5), 125 mM NaCl and 0.1% Tween 20) for 1 h at room temperature and incubated with the appropriate primary antibodies at 4 °C overnight. Antibody recognition was detected with horseradish peroxidase (HRP)-conjugated secondary antibodies for 2 h at room temperature. Finally, protein bands were detected using an enhancement chemiluminescence substrate (Supersignal West Pico; Pierce, Rockford, IL, USA) and quantified using ImageJ software.

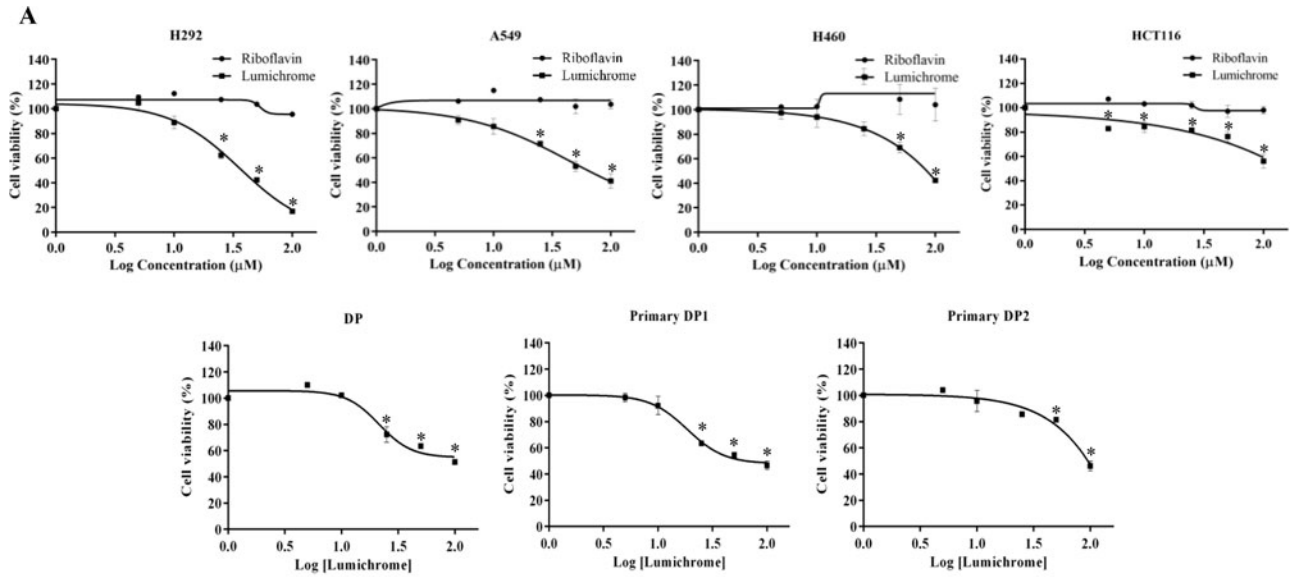
Flow Cytometry Analysis

To determine CD44 and CD133 expression by flow cytometry, H460 cells treated with lumichrome (0, 25, and 50 µM) for 24 h were washed with PBS and incubated in PBS supplemented with 3% BSA for 30 min on ice. After that, cells were incubated in primary antibody for 1 h at 4 °C. The cells were then incubated in Alexa fluor 488-conjugated secondary antibody for 30 min at 4 °C in the dark. After washing, cells were analyzed using Guava easyCyte flow cytometers (Millipore Co., CA, USA).

Statistical Analysis

All data from at least three independent experiment are expressed as mean ± standard deviation (SD). Statistical differences between multiple groups were analyzed using analysis of variance (ANOVA), followed by Turkey's post-hoc test for individual comparisons at the $P < 0.05$ significance level.

A



B

Cells	IC50 (μM)	
	Lumichrome	Cisplatin
Cancer cells		
H292	38.09±1.45	46.50±2.01
A549	53.83±6.13	72.79±2.52
H460	78.79±7.10	56.27±0.83
HCT116	>100	>100
Normal cells		
DP cell lines	>100	>100
Primary DP1	69.79±7.22	45.32±0.93
Primary DP2	95.23±3.99	44.62±0.06

Figure 2. Cytotoxicity effects of lumichrome on human cancer and normal cells. A: Cells treated with lumichrome for 24 h were determined by MTT assay. B: The half maximal inhibitory concentration (IC50) of each cell line at 24 h was calculated. Data represent the mean ± SD ($n = 3$). *Significantly different at $P < 0.05$ versus nontreated control.

Results

Lumichrome Decreased Viability of NSCLC Cells

To evaluate the anti-cancer activity of lumichrome in human cancer cells, we assessed a panel of NSCLC cells (H292, A549, and H460) as well as colorectal cancer cells (HCT116) and normal cells (DP cell lines, primary DP1 and DP2 cells). Lumichrome was extracted from the culture broth of *Streptomyces pseudovenezuelae* SKH1-2 (Figure 1). For comparison, riboflavin, the precursor of lumichrome, and cisplatin, the standard chemotherapeutic drugs for lung cancer therapy, were used at the same concentrations. The results showed that lumichrome exhibited cancer-suppressing activity in a dose-dependent manner in all three of the NSCLC cell lines but was less effective in HCT116 (Figure 2A). Also, the limited anti-cancer activity of cisplatin against colorectal cancer cells was observed with the an IC50 value of more than 100 μM for both cisplatin and lumichrome (Figure 2B). In the case of lung cancer cells, it can be seen that lumichrome exhibited a potential cytotoxic effect as its

IC50 value was substantially lower than those of cisplatin in H292 and A549 cells (Figure 2B). Meanwhile, riboflavin at concentrations from 0 to 100 μM had no anti-cancer activity (Figure 2A). Interestingly, lumichrome was found to have lower toxicity to normal cells compared to cisplatin. These data indicate the promising anti-cancer activity of lumichrome.

Lumichrome Inhibits Clonogenic Growth of NSCLC Cells

Evading growth inhibition and growth in an anchorage-independent condition are among the characteristics of aggressive cancer (29). It is interesting that lumichrome may inhibit growth of lung cancer cells in such a condition. The effect of lumichrome on the clonogenic growth of H292, A549, and H460 cells was examined. As shown in Figure 3A,B, lumichrome at concentrations of 25 and 50 µM significantly inhibited colony formation and growth in H292, A549, and H460 cells. We further determined the effect of lumichrome on the growth of H292, A549, and H460 cells under an anchorage-independent condition. The results showed that lumichrome at concentrations of 10 to 25 μM, 50 μM, and 25 to 50 μM significantly reduced anchorage-independent colony formation in H292, A549, and H460 cells, respectively (Figure 3C,D). These results suggest that lumichrome inhibits clonogenic survival of NSCLC cells.

Lumichrome Induces Apoptosis via p53 Activation

To investigate the apoptosis-inducing effect of lumichrome, cells were treated with lumichrome and apoptotic cell death was determined by Hoechst 33342/PI staining assay. Lumichrome at concentrations of 25–50 μM caused a significant increase in the number of apoptotic cells representing condense and fragmented nuclei in H292 cells compared with nontreated control cells (Figure 4A,B). Consistent with this finding, apoptotic cell death was significantly increased in A549 (Figure 4C,D) and H460 (Figure 4E,F) cells treated with lumichrome at 25–50 µM. It is noticeable that necrotic cell death was not observed in H292, A549, and H460 cells treated with lumichrome, even at high concentrations.

To support the above apoptotic activity of lumichrome, the markers of apoptosis—including activation of cleavage of PARP, caspase-9 and caspase-3—were examined in NSCLC cells treated with lumichrome at 0–50 µM. Western blot analysis showed that the treatment of H292 cells with lumichrome at the concentrations of 25 and 50 µM markedly increased the level of cleaved PARP, while reducing the total intact PARP (Figure 5A,C,E). In agreement with such results, expression of the active forms of caspase-3 and caspase-9 was found to be significantly upregulated in H292 cells treated with lumichrome at 50 µM (Figure 5A,B). In A549 cells (Figure 5C,D), lumichrome at 50 µM significantly induced the cleavage of PARP and activated caspase-9 but did not increase the level of the active form of caspase-3. Meanwhile, expression of the active forms of caspase-3 and caspase-9 was found to be significantly induced in H460 cells treated with lumichrome at 50 µM (Figure 5E,F). These findings support the theory that lumichrome strongly induces apoptosis in NSCLC cells.

One important mechanism of action in currently used anti-cancer drugs is mediating p53-dependent apoptosis (30–32). The activation of p53 in response to drug action leads to the induction of pro-apoptotic protein BAX and reduction of anti-apoptotic proteins BCL2 (5,6). Additionally, BCL2 family proteins are important mediators for chemotherapeutic resistance (33,34). Therefore, the expression of p53, BAX, and BCL2 was examined in NSCLC cells after lumichrome treatment. Lumichrome increased the expression level of p53 and subsequently decreased the expression of BCL2, while that of BAX was not changed in H292, A549, and H460 cells (Figure 5). Taken together, these results provide evidence of the potential of lumichrome to induce apoptosis via activating p53 as well as suppressing the anti-apoptotic BCL2 protein in NSCLC cells.

Lumichrome Suppresses Cancer Stem Cell Signals in Lung Cancer Cells

CSCs are known to be the determining factor in the aggressive behaviors of various cancers (35). For lung cancer, AKT and β-catenin signaling pathways were shown to contribute CSC phenotypes (36,37). Therefore, we determined whether lumichrome could be able to suppress such CSC signals. Cells were treated or left untreated with the compound for 24 h and levels of lung CSC markers CD44 and CD133 were determined. In addition, the corresponding upstream signals, including AKT and β-catenin, were monitored by using western blotting. Figure 6A,B show that the nontreated H460 cells notably expressed CD44 and CD133. The levels of such CSC makers were significantly decreased in response to

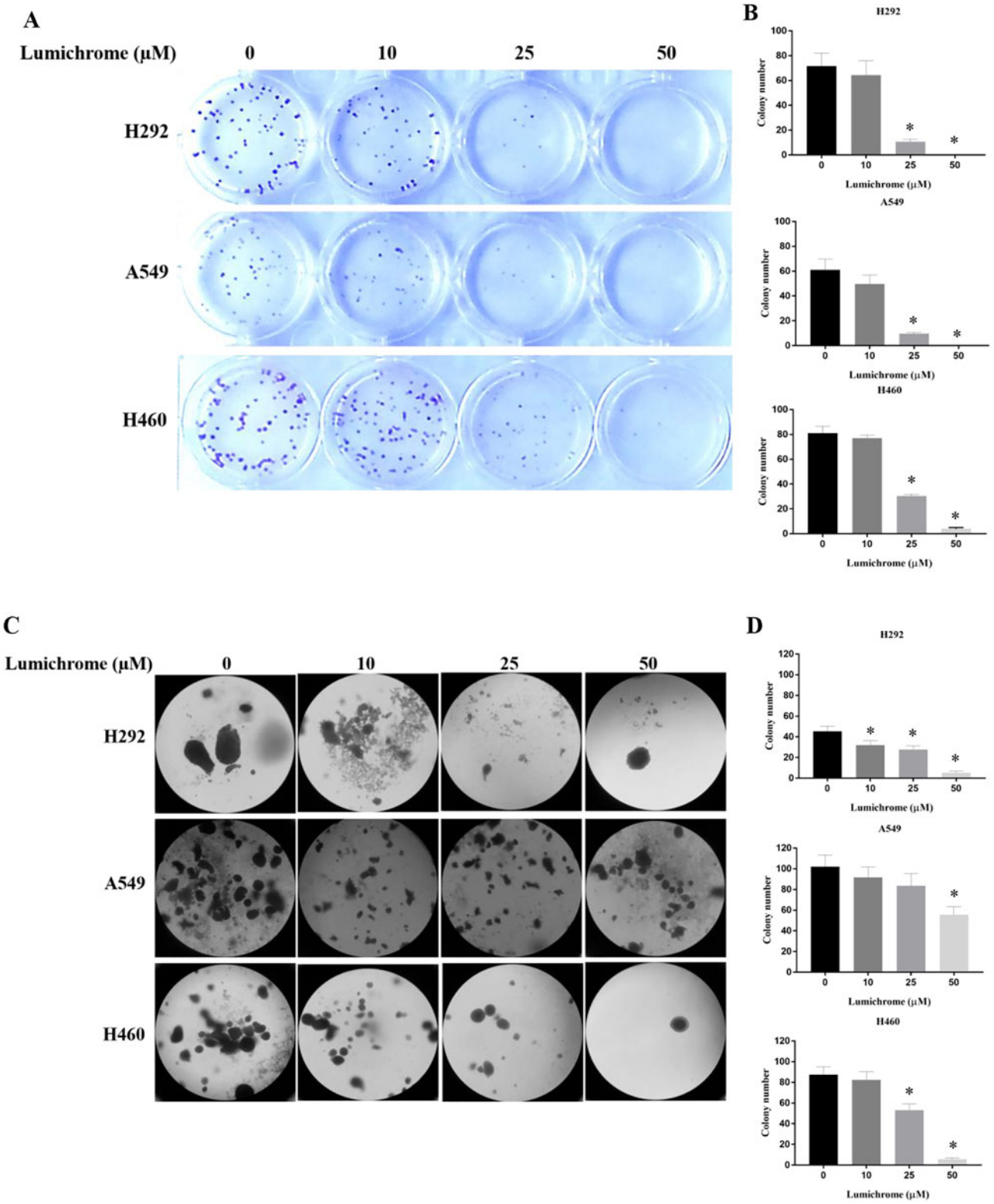


Figure 3. A: Representative figure of colony formation assay, and B: the number of colonies. C: Representative figure of anchorage-independent growth assay, and D: the number of colonies. Data represent the mean ± SD ($n = 3$). *Significantly different at $P < 0.05$ versus nontreated control.

lumichrome treatment, suggesting the CSC-suppressive property of the compound in lung cancer cells. Next, we found a reduction in stem cell maintaining signals after the cells were exposed to lumichrome. Lumichrome caused a reduction in active AKT and β-catenin (Figure 6C,D). This CSC-suppressing activity of the compound was confirmed by counting the CD44 and CD133 positive cells using flow cytometry

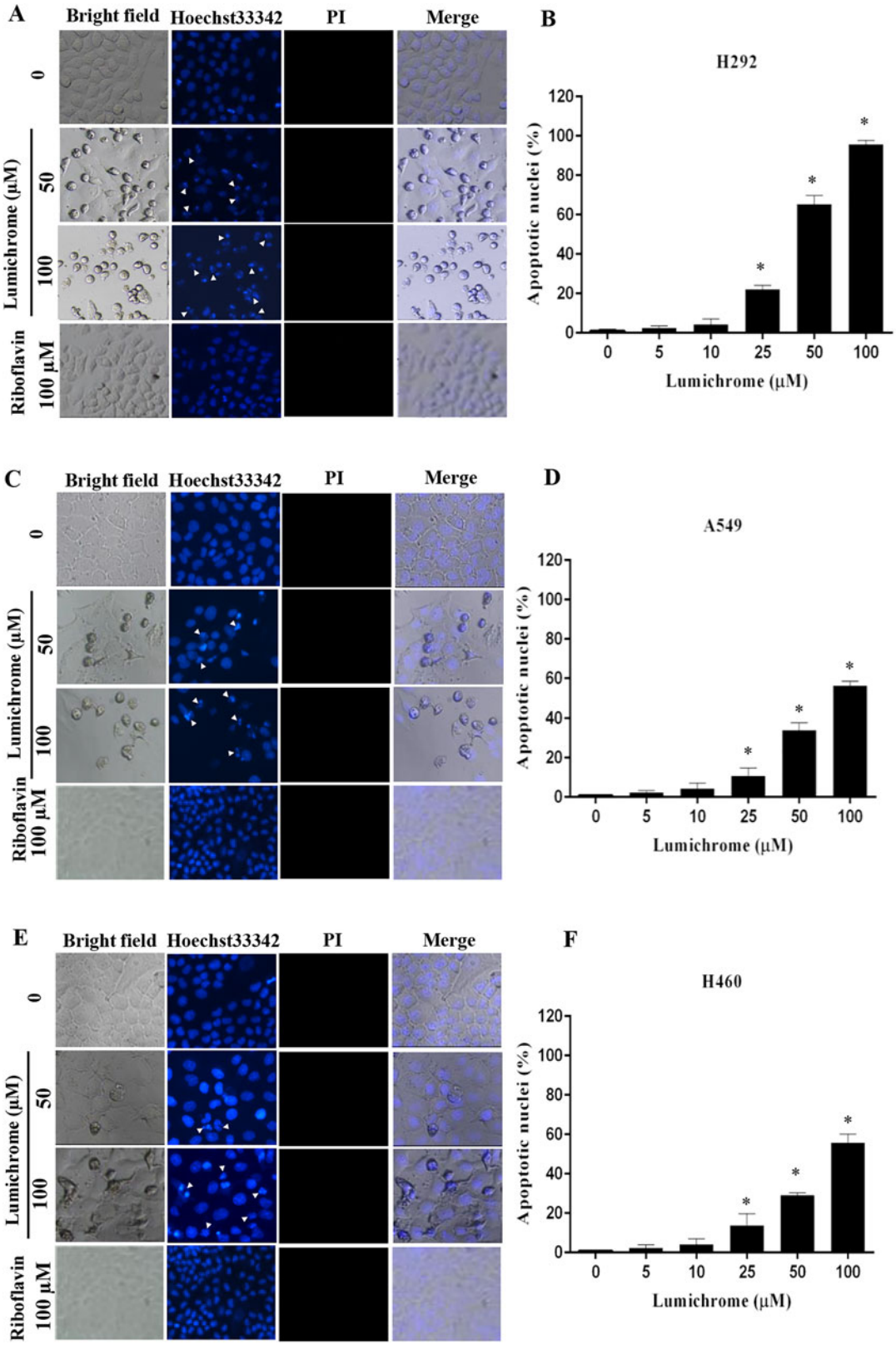


Figure 4. Apoptosis induction by Lumichrome in A: H292, C: A549, and E: H460 was determined by Hoechst33342/PI staining. Arrowheads indicate representative apoptotic nuclei in H292, A549, and H460 cells. The percentage of apoptotic nuclei in lumichrome-treated cells was calculated in B: H292, D: A549, and F: H460. Data represent the mean ± SD ($n = 3$). *Significantly different at $P < 0.05$ versus nontreated control.

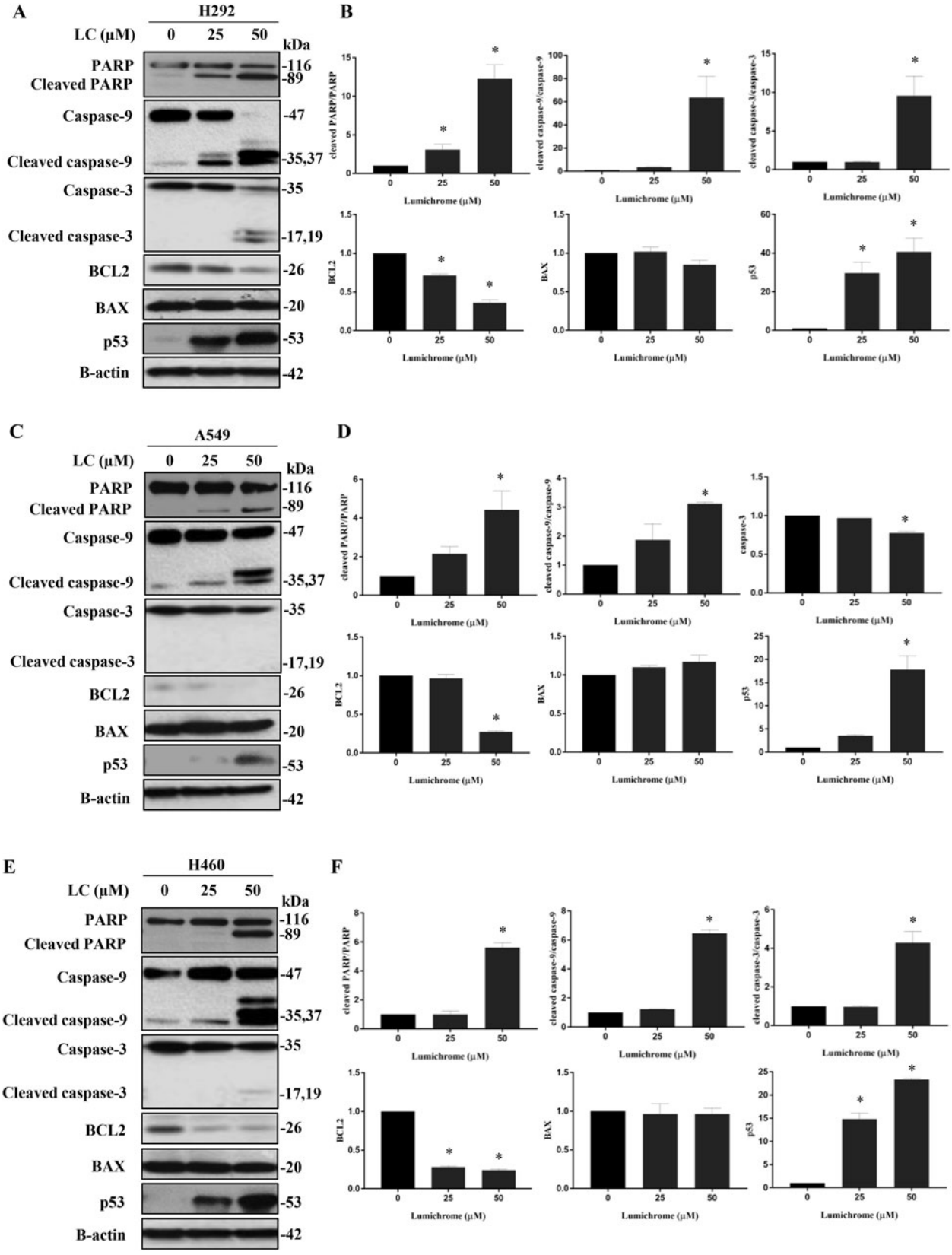


Figure 5. Western blot analysis of apoptosis-associated proteins. Cells were treated with lumichrome for 24 h. A: The expression levels of poly (ADP-ribose) polymerase (PARP), cleaved PARP, caspase-9, cleaved caspase-9, caspase-3, cleaved caspase-3, BCL-2, BAX, and p53 proteins in A: H292, C: A549, and E: H460 cells were determined by western blotting. To confirm equal loading of the samples, the blots were reprobed with the β-actin antibody. Relative protein levels were quantified by densitometry in B: H292, D: A549, and F: H460. Data represent the mean ± SD ($n = 3$). *Significantly different at $P < 0.05$ versus nontreated control.

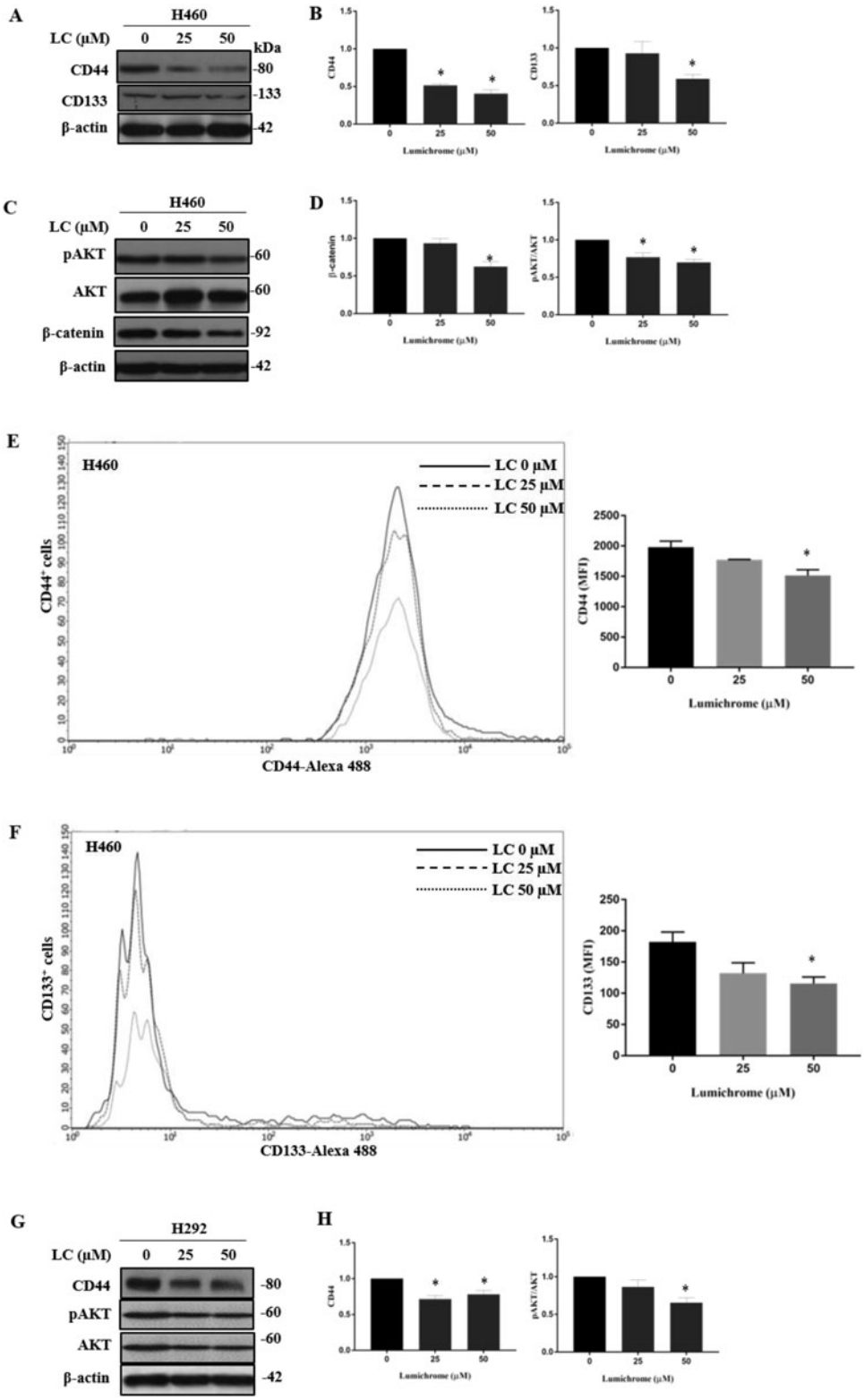


Figure 6. Lumichrome represses cancer stem cell signals. Western blot analysis of apoptosis-associated proteins. Cells were treated with lumichrome for 24 h. A: The expression levels of cancer stem cell markers CD44 and CD133 in H460 cells treated with lumichrome (0–50 μM) were determined by western blotting. The blots were reprobed with the β-actin antibody. B: Relative protein levels were quantified by densitometry. C: Cellular levels of activated AKT (pAKT), total AKT, and β-catenin were determined by western blot analysis. D: The immunoblot signals were quantified by densitometry. E: The $CD44^+$ cells and F: the $CD133^+$ cells were determined by flow cytometry. G: The CD44, pAKT, and AKT levels were determined by western blot analysis in lumichrome-treated H292 cells. H: Relative protein levels were quantified by densitometry.

(Figure 6E,F, respectively). Results indicated that CD44 and CD133 positive cells had significantly declined after lumichrome treatment. We also provide supportive data indicating that lumichrome could reduce the expression levels of CD44 and pAKT in H292 cells (Figure 6G,H). These data reveal the additive effect of lumichrome in overcoming CSCs in lung cancer.

Discussion

The derivative of riboflavin (vitamin B2) is of interest with regard to possible development for anti-cancer approaches. Surprisingly, several studies have reported the possible tumor-promoting activity of riboflavin, a well-known water-soluble diet component. Although, the definite mechanism is not known, studies have indicated that deficiency of riboflavin suppresses the growth rate of cancer in experimental animals and possibly in man (38). In lung cancer, it has been shown that riboflavin can promote cancer proliferation, migration, and invasion (27). Unlike riboflavin, its derivative lumichrome, has anti-cancer activity. We demonstrated here that lumichrome not only inhibited growth of lung cancer cells but it also attenuated CSCs and induced apoptosis. Regarding cytotoxicity, lumichrome seems to be less toxic to normal cells (Figure 2A) as its IC50 for normal dermal papilla cells is about 70–95 μM, while for lung cancer cells it is around 40–80 μM (Figure 2B). Additionally, even though the cancer-killing effects are comparable, the toxic effect of lumichrome is much lower than that of cisplatin, a standard treatment platinum-based drug used in standard treatment for lung cancer. The IC50 of cisplatin in H292 and H460 cells was lower than that for A549 cells indicating higher sensitivity of H292 and H460 cells to cisplatin. The previous report indicated H460 cells were more sensitive to cisplatin than A549 cells (39).

The tumor suppressor p53 protein, encoded by *TP53* gene, has an important role in the regulation of cell cycle, DNA repair and apoptosis (40). Loss of *TP53* gene function by mutations, found in about 50% of cases of NSCLC (41,42), is related to adverse prognosis, tumor progression and drug resistance (43). Previous studies showed p53 gene status regulates chemosensitivity and apoptosis in NSCLC cells treated with various anti-cancer drugs including cisplatin (44, 45). Mutant p53-carrying NSCLC cells (NCI-H1437, NCI-H727, NCI-H441, and NCI-H1299) transfected with the wild-type p53 exhibited increase in chemosensitivity and induction of apoptosis, while mutant p53-transfected H460 cells showed the loss of chemosensitivity and p53-mediated apoptosis in response to camptothecin, etoposide, and cisplatin (44). We found that lumichrome could increase the expression level of p53 protein in H292, H460 and A549 cells that express wild-type p53. The wild-type p53 protein regulates apoptosis by acting as transcription factor for pro-apoptotic genes including BAX and BCL-2 (5,6). Our study found that BCL-2 expression was decreased, while that of BAX was not changed by lumichrome in lung cancer cells. These data suggest that p53 and BCL-2 may have a role in the regulation of lumichrome-induced apoptosis in certain NSCLC cells (Figure 5).

The growth of cancer cells in an anchorage-independent manner has been linked to the ability of cancer cells to survive during metastasis (29,46). We found that treatment of lung cancer cells with lumichrome significantly reduced cell survival and growth in a detached condition (Figure 3C), suggesting that the compound may in part inhibit the metastasis of cancer cells by decreasing the survival of the cells with a detached status. Likewise, CSCs were shown to be the cells which have a strong ability to survive during metastasis and give rise to the new tumors at distant parts of the body (8,47,48). CSCs are defined as unique cells with the capability of self-renewal and differentiation that is required for new tumor initiation. Further evidence has also demonstrated that CSCs are responsible for chemotherapeutic failure, cancer dissemination, and relapse (9,49). Recently, CSC-targeted therapy has been suggested as a new and promising tool which could be used in eliminating malignant cancers (50). Several bioactive compounds have been reported for their CSC-suppressive activity and a certain number of them are undergoing clinical trials (12,51). We have demonstrated herein for the first time that lumichrome has the ability to suppress CSCs in lung cancer (Figure 6).

An attempt has been made to identify the molecular signals underlying cancer cell stemness. Certain signals found in normal stem cells were found to display pivotal roles in maintaining CSC properties. Activation of the AKT pathway has been highlighted as a CSC-supporting signal in many malignancies, including breast cancer (25) and lung cancer (52). The theory that AKT increases the stem cell transcriptional activity of β-catenin has been demonstrated in many cancer cells (21,36,37). In detail, AKT phosphorylates β-catenin at the position of Ser552 and such a phosphorylation of β-catenin causes its disassociation from the inactive complex and increases the accumulation

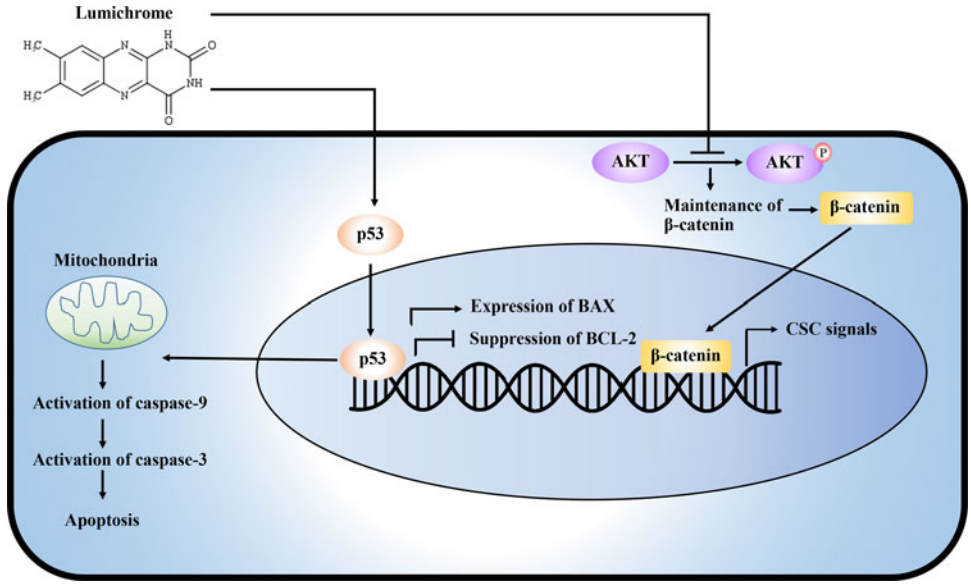


Figure 7. Schematic representation of anti-cancer activity of lumichrome on lung cancer cells. Lumichrome activates p53, which in turn upregulates pro-apoptotic BAX and decreases anti-apoptotic BCL-2 proteins. The imbalance of BCL-2 family proteins activates mitochondrial apoptotic machinery by activating caspase-9 and caspase-3, resulting in apoptosis. In addition, lumichrome is able to suppress AKT function, which maintains β-catenin level and function. The decrease in AKT and β-catenin causes the depletion of CSC properties.

of β-catenin in cytosol and nucleus. This phosphorylation of β-catenin renders its transcriptional activity so it functions as a co-transcription factor of TCF/LEF and consequently regulates expression of proteins facilitating stem cell functions (53,54). Furthermore, AKT was shown to increase the cellular level of β-catenin by interfering with GSK3β-dependent β-catenin degradation. The cellular level of β-catenin is diminished by the function of GSK3β. Generally, GSK3β phosphorylates β-catenin and subjects it to proteasomal degradation. AKT inhibits such a function of GSK3β by phosphorylating GSK3β at serine 9, resulting in the increase in β-catenin stability (55).

Collectively, we have provided sufficient data supporting the anti-cancer activity of lumichrome in several approaches, including growth inhibition and CSC suppression. Not only could the compound inhibit growth in normal and anchorage-independent conditions, but lumichrome directly mediated cancer cell apoptosis through p53-dependent mechanisms (Figure 7). In addition, lumichrome was shown to inhibit CSC in lung cancer cells by attenuating AKT/β-catenin signals, which may be further developed for CSC-targeting approaches.

Acknowledgment

The authors would like to thank the proofreader Krich Rajprasit, International College for Sustainability Studies, Srinakharinwirot University.

Disclosure Statement

The Authors declare that there are no conflicts of interest in regard to this study. The Authors alone are responsible for the content and writing of this article.

Funding

This work was supported by the Ratchadaphiseksompot Fund for Postdoctoral Fellowship, Chulalongkorn University and the grant for International Research Integration: Chula Research Scholar, Ratchadaphiseksomphot Endowment Fund.

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