약학 석사학위 논문

Screening of Natural Products for the Development of Anti-cancer Agents in Non-Small Cell Lung Cancer (NSCLC).

폐암 세포에서 항암제 개발을 위한 천연물 스크리닝

2014년 2월

서울대학교 대학원
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이 논문을 약학석사 학위 논문으로 제출함

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ABSTRACT

Screening of Natural Products for the Development of Anti-cancer Agents in Non-Small Cell Lung Cancer (NSCLC).

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Non-small cell lung cancer (NSCLC) is one of main causes of human deaths, because current therapeutic approaches have failed to improve survival rates of patient with NSCLC. To develop effective anti-cancer therapeutic strategies, constant efforts have been made. Natural products have served as one of a main sources for discovering novel anticancer drugs. It has been reported that about 60% of approved cancer chemotherapeutic agents were derived from natural products.

In this research, we evaluated a number of natural products derived from various medicinal plants for their potential as anticancer chemopreventive and/or therapeutic drugs. The effects of natural products on proliferation,

survival and apoptosis were assessed in NSCLC cells, premalignant, and normal human bronchial epithelial (HBE) cell lines. The MTT assay, anchorage-dependent and -independent colony formation assays and fluorescence-activated cell sorting (FACS) assays, and western blot analysis were used to analyze the proteins involved in cell survival and apoptosis. We also confirmed the *in vivo* inhibitory effects of a couple of natural products on the growth of H1299 NSCLC xenograft tumors established in NOD/SCID mice. The potential mechanisms, mediating the anti-cancer effects of a potent drug were assessed by using the RTK signaling antibody array kit and by using PCR, western blot and luciferase assays. Several natural products exhibited significant inhibitory effects on the proliferation of premalignant HBE and NSCLC cells with minor influence on normal HBE cells at similar concentrations. Among these products, a drug (compound **1**) with these capacities effectively inhibited the IGF-IR signaling pathway by suppressing IGF-2 transcription. We also observed that compound **1** suppressed the growth of NSCLCL xenograft tumor in mice. In summary, our data elucidates the potential of compound **1** as an anticancer chemopreventive and/or therapeutic agent against NSCLC.

Key words: Lung cancer, Natural product,

Student number : 2012-21612

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I. INTRODUCTION

Non-small cell lung cancer (NSCLC) leads the cause of cancer-relate incidence and mortality rate in the United States and many other parts of the world. In 2013, 159,480 deaths, about 27% of all cancer deaths, are expected to occur (Society, 2013). Despite of constant efforts and advances in therapeutic strategies, overall survival rate and 5-year survival rates have not improved [1]. Therefore, cancer prevention is one of strategies to alleviate the results of disease. Several natural compounds have been discovered and developed as chemopreventive agents [2-4]. However, side effects and resistance of lung cancer cells to agent limited their potential use in clinic [5, 6]. Therefore, we have tried to discover novel agents that have potential as a novel chemopreventive/therapeutic agent in NSCLC.

We screened 511 natural products *in vitro* and *in vivo* model systems. Out of those, we identified 14 natural products which inhibited growth of premalignant HBE and NSCLC cells with minimal toxicity to normal HBE. Among them, compound **1**, has been found to exhibit chemopreventive and therapeutic activities both *in vitro* and *in vivo* in model sytems.

Compound **1** is a phytochemical which is in a class of steroid saponin. Compound **1** is abundantly found in legumes and yams, widely distributed in asian countries. Compound **1** is in a family of diosgenin and has been used traditionally for preventive or therapeutic medicine against several ailments, including cancer, diabetes, high cholesterol and inflammation. However, there has been no report that addressed anti-cancer effects of compound **1**. In our efforts for searching anti-cancer agents from natural products, the potential

chemopreventive/therapeutic effects of compound 1 on anti-cancer responses were evaluated.

In order to investigate potential of compound 1 as chemopreventive/ therapeutic drug, we assessed the changes in compound 1- treated premalignant HBE and NSCLC cell lines and observed suppression of the IGF-IR signaling pathway.

Insulin-like growth factor (IGF) axis contains ligands, receptors, substrates, and ligand binding proteins. And it has been associated with the risk of lung cancer. Insulin-like growth factor (IGF)-I receptor (IGF-IR) signaling and the levels of its ligand (IGF2) are known to play a role in cell survival and transformation [7-12]. The ribosomal s6 kinase (RSK) is a family of protein kinases involved in signal transduction. And it is known to constitutively activated in various cancer cells [13]. There are two subfamilies in rsk, p90S6K and p70S6K. Between them, compound 1 strongly regulated p70S6K. The kinase activity of this protein leads to an increase in protein synthesis and cell proliferation. p70S6K is in a signaling pathway that includes the Akt/mammalian target of rapamycin (mTOR). Akt/mTOR pathway is known to typically activated by stimulation of growth factors, then the downstream of akt, mTOR leads to p70 S6K activation. The Akt/mTOR pathway plays a major role in regulation of mRNA translation, many of which encoded proteins are involved in cell proliferation, growth and angiogenesis [14]. Inactivation of IGF-IR by various methods, such as gene disruption, neutralizing antibodies, or dominant-negative mutants, has been shown to block tumorigenesis activites [15].

And the expression level of IGF2 in high-grade dysplasia was significantly

higher than normal epithelium, hyperplasia and squamous metaplasia [16]. Those findings led us IGF-IR signaling pathway could be an effective anti-cancer target in lung cancer.

In the present study, the anti-cancer activities of compound **1** and its possible mechanism of action were investigated in both *in vitro* cell culture and *in vivo* animal models.

II. METARIALS AND METHODS

A.Materials

1. Reagents and antibodies

RPMI 1640 medium, fetal bovine serum(FBS), antibiotics-antimycotics solution and trypsin-EDTA were purchased from Welgene biopharmaceuticals (Daegu, Republic of Korea). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT) was purchased from MP Biomedical(Santa Ana, CA, USA) and other chemicals were purchased from Sigma(St. Louis, MO, USA) unless otherwise indicated.

2. Compounds

Compounds were provided by Prof. Kang Sam Sik (College of Pharmacy, Seoul National University, Seoul 151-742, Republic of Korea).

3. Cell and Cell culture

H1299, A549, H226, obtained from the American Type Culture Collection (ATCC), were cultured in RPMI 1640 supplemented with 10% FBS and 1% antibiotics-antimycotics. Normal HBE (NHBE) cells were purchased from Clontech(Palo Alto, CA) and maintained according to the manufacturer's recommended protocol. BEAS-2B cells, an HBE cell line immortalized with a hybrid adenovirus/simian virus 40(obtained from Dr. A. Klein-Szanto, Fox Chase Cancer Center, Philadelphia, PA) were previously used to derive

premalignant HBE cell, 1198, as follows [17, 18]. Cells were incubated at 37℃, with 5% CO_2 in a humidified atmosphere

B. Methods

1. MTT assay

Inhibition of cell growth and proliferation by compounds was used to assess by MTT assay. Briefly, 2000-4000 cells seeded onto each well in 96-well plates. 24 h after; seeding, change fresh medium with different concentration of drugs or DMSO (dimethyl sulfoxide) for control. After 3 days, 500 μg/mL of MTT solution was added to each well and incubated at 37℃ for 4 h. When each cells stained clearly, remove medium. DMSO was added to each well to dissolve generated formazan and the plates were measured at 570 nm using a spectrometer.

2. Anchorage - dependent and - independent assay

Anchorage - dependent assay was done by seeding NSCLC (200 cells per well) cells into 6-well plates (SPL Life science, Pocheon, Republic of Korea). Every 3 days, cells were treated different concentrations of compound **1**. After 10-15 days, in a humidified atmosphere with 37℃ and 5% CO_2 , colonies were fixed in 99% MeOH and stained with 0.01% crystal violet solution and then counted.

For the anchorage-independent assay, as a bottom agar, 1 mL of medium containing 1% SeaPlaqueGTG agaros (Lonza, Rockaland, ME) was added and left to solidify at room temperature. 500-1000 cells, resuspended in 0.5 mL of medium containing 0.4% agar, were plated as triplicate. Every 3days,

medium containing different concentrations of compound **1** were changed. After 10−15 days, colonies were stained with 500 μg/mL MTT for 3 h. Image of colonies were taken by Fuji LAS−3000 imaging system and counted with Image J software.

3. FACS analysis

NSCLC and NHBE cells were plated at a concentration of 1×10^6 cells/100 mm plates. The next day, cells were treated with either DMSO as a control or various concentrations of natural products. All cells (non−adherent and adherent) were harvested, fixed with 100% methanol, stained with 50 μg/mL propidium iodine, and subjected to flow cytometric analysis to determine the percentage of cells in phase of cell cycle. Flow cytometric analysis was performed using a BD FACS Calibur.

4. Western blot analysis

To prepare total protein, cells were lysed with ice−cold lysis buffer(1% NP−40, 0.25% sodium deoxycholate, 1 mM EDTA, 150 mM NaCl, 50 mM Tris−HCl[pH7.4], 1% Triton X−100, 10% Glycerol) including a complete protease inhibitor cock tablet(Roche diagnostics, Mannheim, Germany) and phosphatase inhibitors (Na_3VO_4 1 mM, NaF 100 mM, NaPP 10 mM). Concentration of lysates was determined by using a biochinconinic acid (BCA) assay kit (Pierce Biotechnology, Rockford, IL, USA). Equivalent amounts of protein were loaded using sodium dodecyl sulfate (SDS)−polyacrylamide gels(6−15%). After transferred to a polyvinyl difluoride (PVDF) membrane, membrane was rocked with 3% BSA in Tris−buffered saline 0.1% Tween−

20 (TBST) for 1 h. Membrane was incubated for 1 h at room temperature or 12 h at 4℃. The membrane was then washed 3 times per 1 h with Tris-buffered saline (TBS) containing 0.1% Tween20 (TBST). The membrane was incubated with appropriate secondary antibody for 1 h at room temperature and washed again in same way. And the protein-antibody complex was visualized by ECL solution (femto) (Thermoscientific INC, Bremen, Germany)

5. Caspase 3&7 enzyme activity

NSCLC and NHBE cells were plated at a concentration of 1×10^6 cells/100 mm plates. The next day, cells were treated with either DMSO as a control or various concentrations of natural products. All cells (non-adherent and adherent) were harvested, and apoptotic cells were identified with CV-Caspase 3&7 Detection Kit (Enzo Lifescience) following protocols provided by the manufacturer.

6. RT-PCR

Total RNA was isolated from cells with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. First strand cDNA was synthesized with the PrimeScript 1^{st} strand cDNA synthesis kit (TaKaRa, Dalian, China) using 1 μg of total RNA according to its protocol. cDNA template was used to amplify genes by RT-PCR with gene-specific primer sets. The amplification products were separated in a 1.5% agarose gel by electrophoresis, and visualized by RedSafeTM nucleic acid staining solution (Intron Biotechnology, INC, Korea).

7. Animal xenograft studies; tumor cell implantation

The in vivo antitumor efficacy of compound **1** was tested in a H1299 xenograft model. NOD-SCID mice (Central Lab. Animal Inc., Seoul, Korea) were subcutaneously inoculated in the flank with 5×10^6 H1299 cells.

Tumor volume was calculated by using a digital caliper. After 2weeks, at tumor size of 100 mm^3, i.p injection of compound **1** was performed.

III. RESULTS

1. Screening of natural products.

To screen natural products compounds, the MTT assay was conducted at 10 μM concentration. Firstly, we tested on Non-small-Cell-Lung-Cancer (NSCLC) line, H1299. Compounds which lowered cell viability by less than 60% were chosen (Table 1). Second, we checked it on Normal Human bronchial epithelial (HBE) cell line, HBEC1 and BEAS2B. Every compound's H1299 cell viability and HBEC1 and BEAS 2B cell viability were compared. Those natural product compounds that have higher Normal HBE cell's viability than NSCLC's, which means lower toxicity to normal cell, were properly selected (Table 2). Then it was evaluated on premalignant cell line and NSCLC line, A549 and H226 (Table 3, Table 4).

Table 1. Screening of natural products (Screening on H1299 cell line)

Classification.	H1299 Cell Viability(%)					Total No.
	0-20(%)	20-40(%)	40-60(%)	60-80(%)	80- (%)	
1. Flavonoids	2	2	7	99	86	196
2. Iridoids	0	0	0	2	14	16
3. Monoterpenoides	0	0	1	0	8	9
4. Sesquiterpenoides	0	1	0	1	5	7
5. Diterpenoides	0	2	1	2	8	13
6. Triterpenoides	0	0	2	6	58	66
7. Steroid Sapogenins	0	0	0	3	4	7
8. Triterpenoid Saponins	0	0	2	5	26	33
9. Steroid Saponins	4	0	0	0	4	8
10. Lignans	0	1	1	3	16	21
11. Coumarins	0	0	0	2	25	27
12. Stilbenoids	0	0	0	0	1	1
13. Alkaloids	0	1	1	1	38	41
14. Phenolics	0	1	9	15	15	40
15. Steroids	0	0	0	6	6	12
16. Ceramides, Cerebrosides	0	0	1	4	1	6
17. Others	0	0	0	0	8	8

Table 2. Screening of natural products (Screening on HBE cell line)

Classification.	HBEC1/ BEAS2B Cell Viability(%)					Total No.
	0-20(%)	20-40(%)	40-60(%)	60-80(%)	80- (%)	
1. Flavonoids	1	5	0	1	7	14
3. Monoterpenoides	0	0	0	0	1	1
4. Sesquiterpenoides	0	0	0	1	0	1
5. Diterpenoides	0	1	0	2	0	3
6. Triterpenoides	0	1	1	0	0	2
8. Triterpenoid Saponins	0	0	1	0	1	2
9. Steroid Saponins	0	0	1	3	0	4
10. Lignans	0	0	1	1	0	2
13. Alkaloids	0	0	2	0	0	2
14. Phenolics	0	0	5	1	3	9
16. Ceramides, Cerebrosides	0	0	0	0	1	1

Table 3. Screening of natural products (Screening on premalignant cell line)

Classification.	1198 Cell Viability(%)					Total No.
	0-20(%)	20-40(%)	40-60(%)	60-80(%)	80- (%)	
1. Flavonoids	0	1	1	2	4	8
3. Monoterpenoides	0	0	0	0	1	1
4. Sesquiterpenoides	0	0	0	1	0	1
5. Diterpenoides	0	1	1	0	0	2
9. Steroid Saponins	3	1	0	0	0	4
10. Lignans	0	0	1	1	0	2
13. Alkaloids	0	0	2	0	0	2
14. Phenolics	0	1	1	2	0	4

Table 4. Screening of natural products (Screening on NSCLC cell line)

Classification.	A549 / H226 Cell Viability(%)					Total No.
	0-20(%)	20-40(%)	40-60(%)	60-80(%)	80- (%)	
1. Flavonoids	0	1	1	0	6	8
3. Monoterpenoides	0	0	0	0	1	1
4. Sesquiterpenoides	0	0	1	0	0	1
5. Diterpenoides	0	1	0	0	1	2
9. Steroid Saponins	1	3	0	0	0	4
10. Lignans	0	0	0	0	2	2
13. Alkaloids	0	0	2	0	0	2
14. Phenolics	0	3	1	0	0	4

2. Effects of compound 1 on the proliferation of normal human bronchial epithelial (NHBE), premalignant and non-small cell lung cancer (NSCLC) cell line.

To determine whether compound 1 could be a potential anti-lung cancer agent, we first examined its effects. To investigate sensitivity of compound 1, we tested the effects of compound 1 on NHBE, premalignant and NSCLC cell line. The growth of cell lines was inhibited by dose-dependent manner. A MTT assay revealed that compound 1 had minimal effect on the growth of NHBE cells. Premalignant and NSCLC cell line were more sensitive to compound 1 (Fig. 1). To further characterize the growth properties of NSCLCs, we investigated the effect of compound 1 on anchorage-independent growth. Anchorage-independent assay demonstrated that compound 1 suppressed ability of colony formation by dose-dependent manner (Fig. 2).

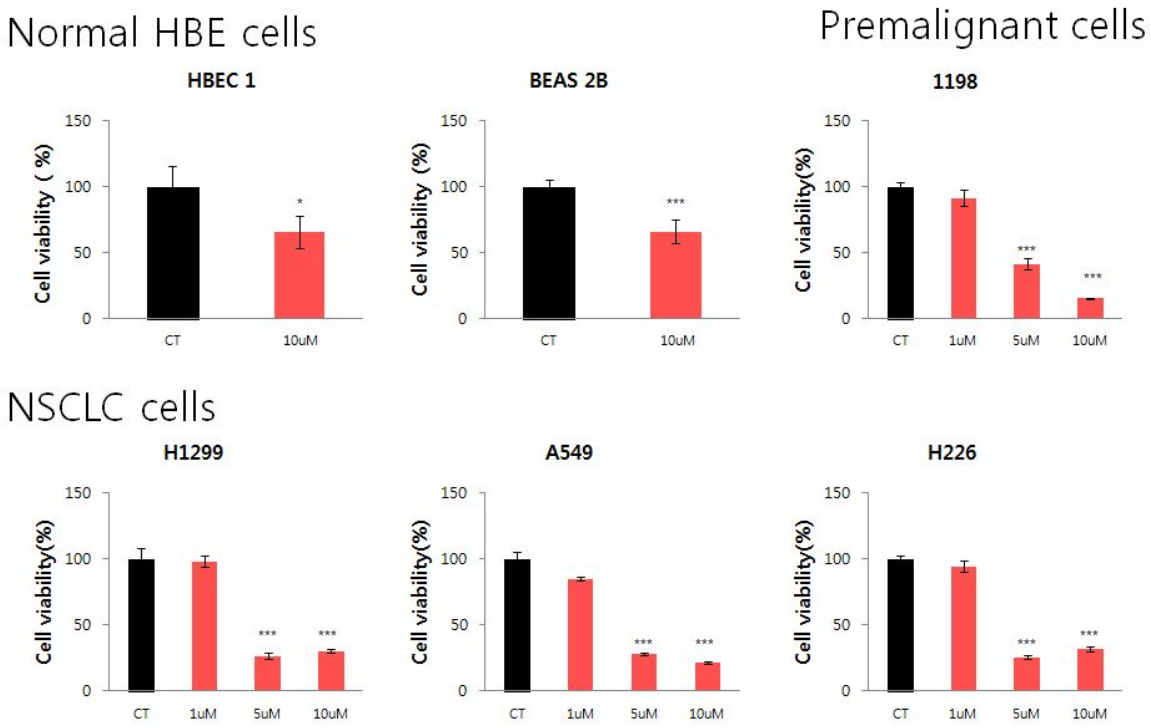


Figure 1. Effects of compound 1 on the proliferation of normal human bronchial epithelial (NHBE), premalignant and non-small cell lung cancer (NSCLC) cell line.

NHBE, premalignant and NSCLC cells were seeded into 96-well culture plate and allowed to adhere over night. The next day, the cells were treated with various concentrations of compound 1 or dimethyl sulfoxide (DMSO) as a control. Cell proliferation was assessed by the 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) assay after 3 days. Results were expressed as percent cell proliferation relative to the proliferation of DMSO-treated cells (Control). Each bar represents the mean value of four identical wells from a representative single experiment.

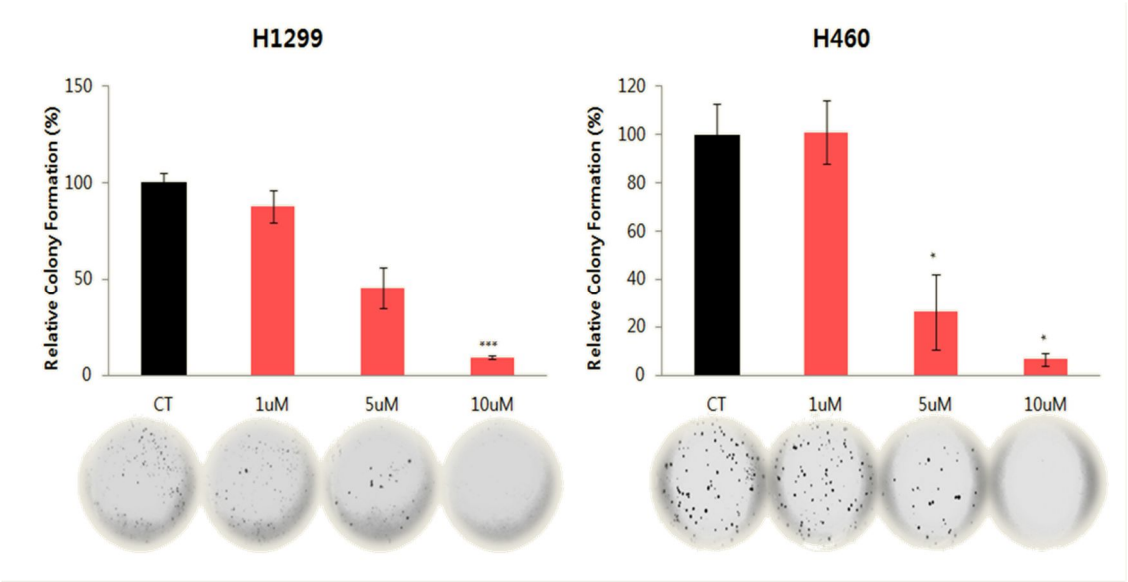


Figure 2. Effect of compound 1 on anchorage-independent colony formation

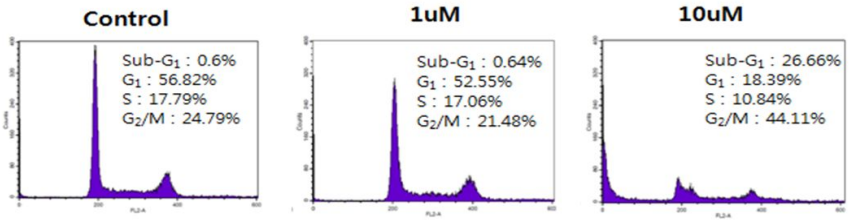
NSCLC cells were grown in soft agar for 10 days+/-, treated with increasing concentrations of compound 1. Anchorage-independent growth was assessed by counting individual colonies in triplicate.

3. Regulation of apoptosis by compound 1.

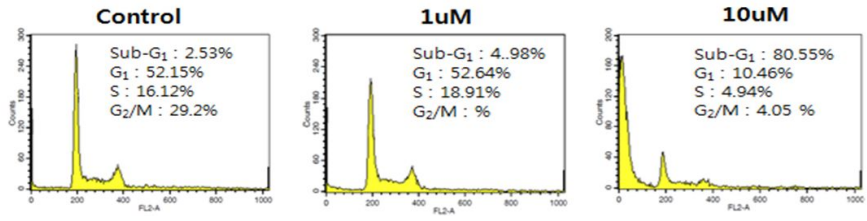
Because the cells that accumulate in the sub-G1 phase of the cell cycle, we hypothesized that compound **1** may have inhibited growth by inducing apoptosis. We confirmed that premalignant and NSCLC cell lines were more sensitive to compound **1** than NHBE cell line by using FACS (Fig. 3). We next assessed the apoptosis - related enzymes (caspase-3, caspase-7 and PARP). There was increase in the cleavage of the poly(ADP-ribose) polymerase(PARP) in NSCLC cells treated with compound **1** for 12 h and 24 h. And we also confirmed that caspase 3 & 7 enzyme activity was increased in the cells treated with compound **1** (Fig. 4).

Normal cell

HBEC1

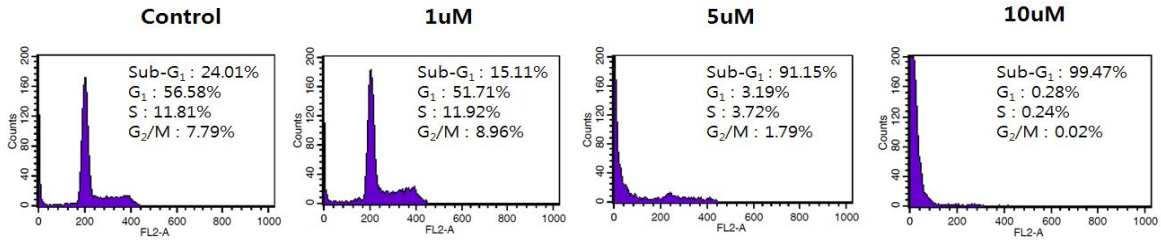


BEAS2B



NSCLC

H1299



A549

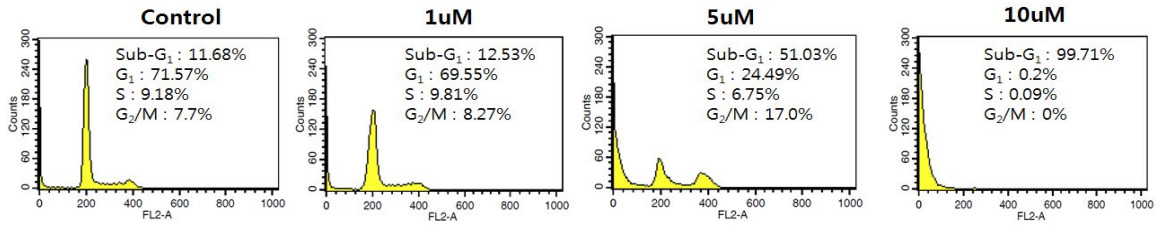


Figure 3. Regulation of cell cycle by compound 1.

Effects of compound **1** on cell cycle distribution of NHBE and NSCLC cell lines. Cells were treated with DMSO (Control) or the indicated concentrations of compound **1** for 24 h were analyzed for DNA content (propidium iodide uptake) and for percentage of cells in specific phases of the cell cycle by flow cytometry as described.

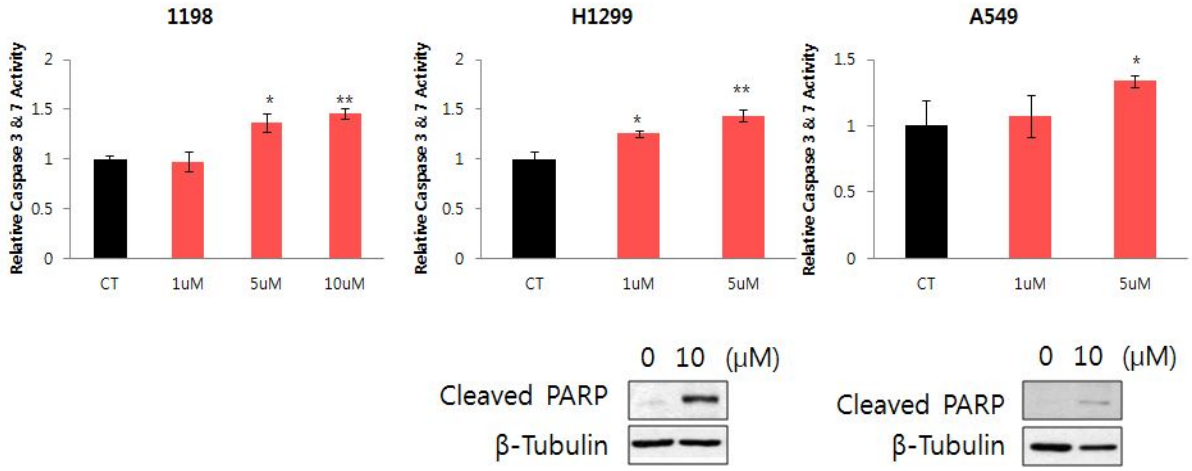


Figure 4. Regulation of apoptosis by compound 1

Premalignant and NSCLC cells were treated with DMSO or indicated concentrations of compound 1 for 24 h. The expression of PARP was analyzed by immunoblotting. Caspase 3&7 activity was detected by using CV – Caspase 3&7 detection kit (BML-AK-118, ENZO).

4. Inhibition of IGF-IR signaling activation by compound1 by regulation of ligand expression.

To investigate changes that are associated with compound **1**, we used receptor tyrosine kinase signaling antibody array kit. As shown in Fig. 5, pp70 S6K was down-regulated in compound **1** treated H1299. We carried out RT-PCR and western blot to confirm level of phospho-p70 S6K. And we identified that IGF-2 ligand and its receptor, IGF-1R were related (Fig. 7, Fig. 8) to phospho-p70 S6K down regulation.

These observations suggest that IGF-2 regulation mediated IGF-1R signaling pathway decrease may be associated with action mechanism of compound **1**.

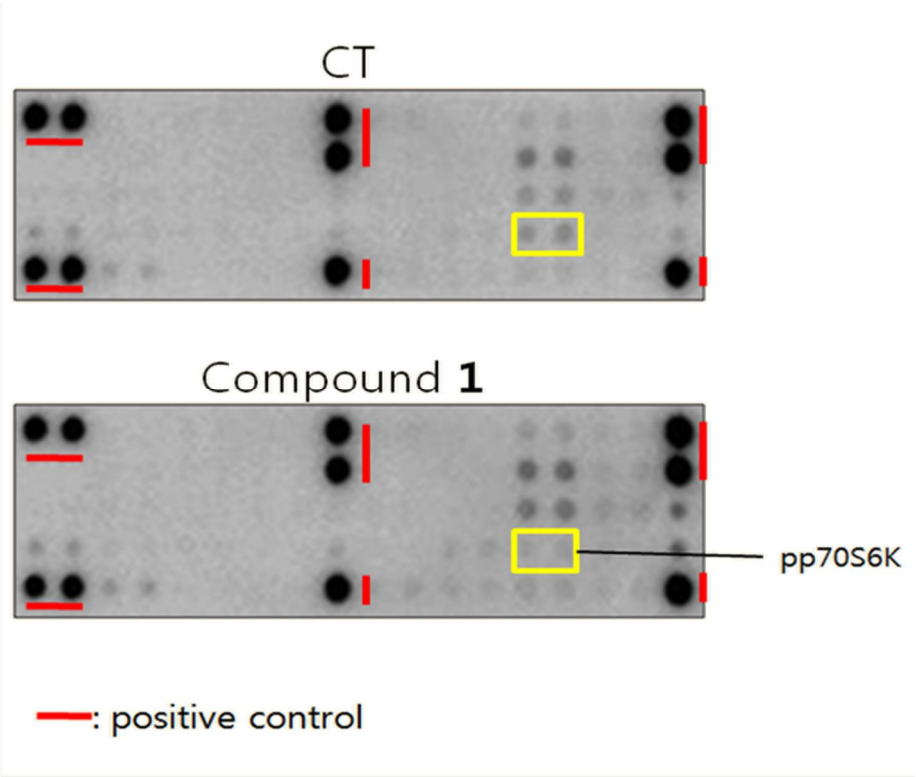


Figure 5. Treatment of H1299 with compound 1 decrease phosphorylation of p70S6K.

NSCLC cell was treated with DMSO or indicated concentration of compound 1 for 24 h. compound 1 decrease phosphorylation of p70S6K as detected by PathScan® RTK Signaling Antibody Array Kit #7982(Cell signaling).

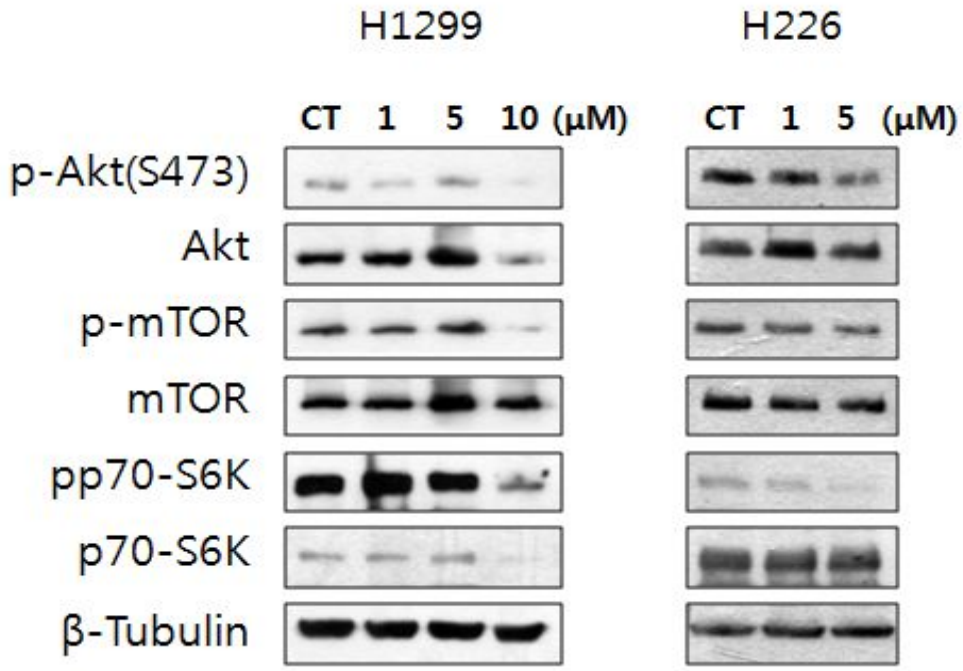


Figure 6. Effect of compound 1 in Akt/mTOR pathway.

NSCLC cells were treated with DMSO or indicated concentration of compound 1 for 24 h. The expression of protein level was measured by western blot analysis.

A. B.

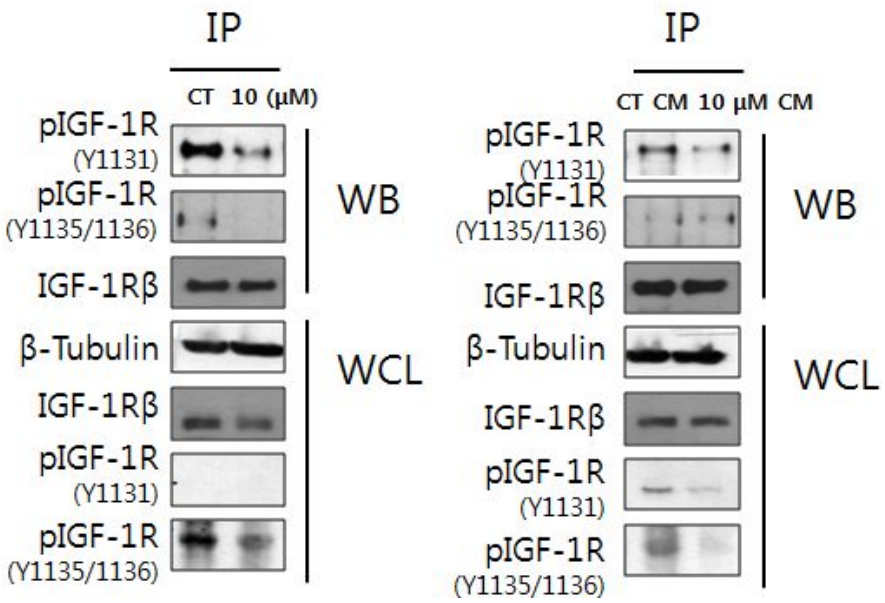


Figure 7. Compound 1 decreases IGF-IR signaling.

A. H1299 was treated with compound 1 or control for 24 h before harvesting for immunoprecipitation. Whole-cell extracts from H1299 cells that were treated compound 1 or control were immunoprecipitated with an anti-IGF-IR antibody, and then proteins in the immunoprecipitates were examined by western blot analysis with antibodies against IGF-IR or pIGF-IR.

B. H1299 was treated with conditioned medium from compound 1 treated H1299 or control medium for 30 min before harvesting for immunoprecipitation. Whole-cell extracts from H1299 cells that were treated with conditioned medium for 30 min were immunoprecipitated with an anti-IGF-IR antibody, and then proteins in the immunoprecipitates were examined by western blot analysis with antibodies against IGF-IR or pIGF-IR.

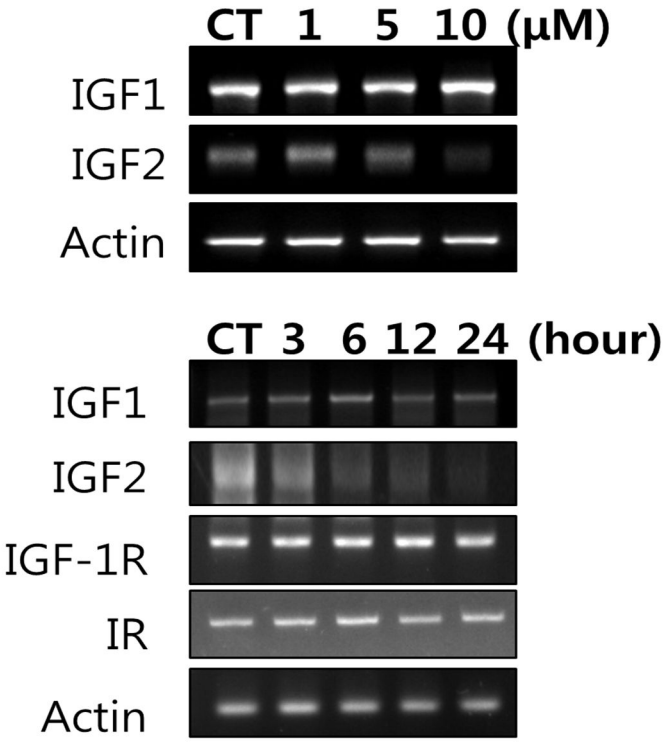


Figure 8. IGF-1R signaling pathway regulated by compound 1- mediated IGF2 down regulation.

H1299 was treated with DMSO or indicated concentration of compound 1 for 24 h. The expression of mRNA level was measured RT-PCR in H1299 cell line.

5. Antitumor effect of compound 1 in tumor xenograft model.

To determine whether compound **1** had anti-tumor activities *in vivo*, we tested the effect of compound **1** on NSCLC growth using H1299 xenograft tumors established in NOD-SCID mice. The mice were then treated with 20 mg/kg of compound **1** or vehicle for 19 days (Fig. 9). Tumor size was measured every 2-3 days for 19 days. Tumor growth in mice that received compound **1** was statistically significantly different from tumor growth in the control groups. At day 19, the mean tumor volume in mice that received compound **1** was approximately 37.9% of that in the mice received vehicle. Thus, these data suggest that compound **1** have antitumor activities in H1299 xenograft model.

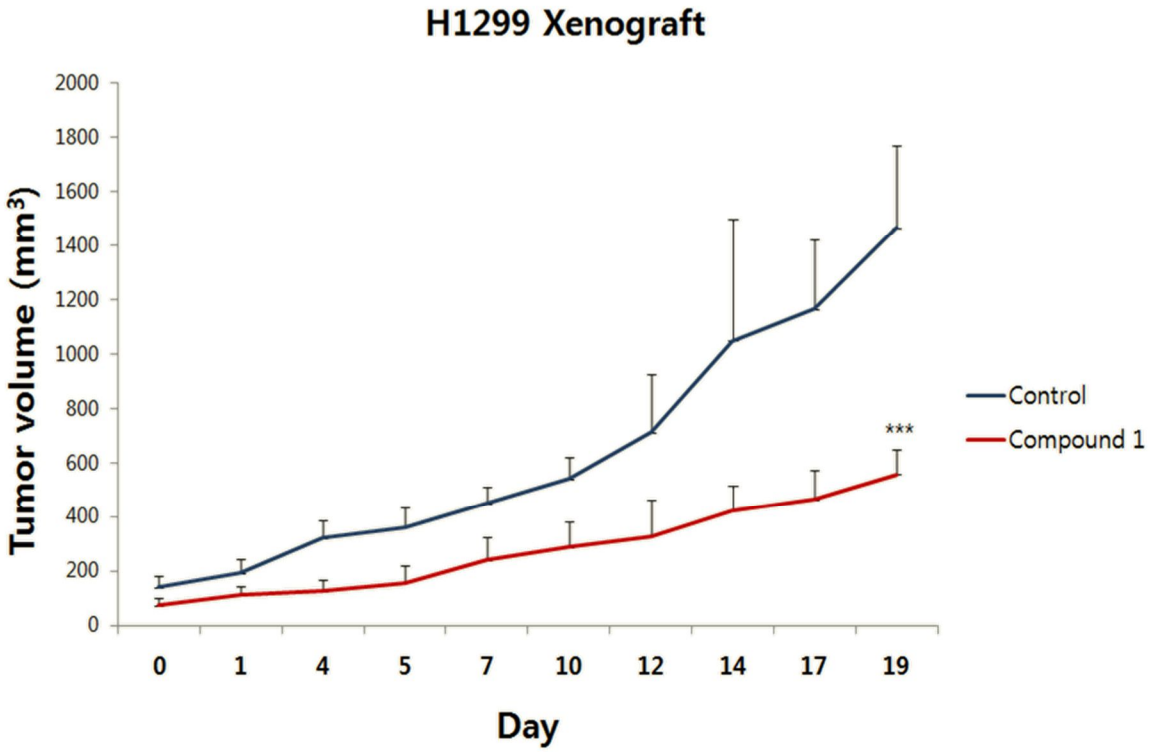


Figure 9. Compound 1 suppresses tumor growth in H1299 xenograft model.

Mice bearing H1299 xenograft tumors were administrated with vehicle (control) or compound 1. Tumors were measured every 2-3 days. Results were expressed as mean tumor volume relative to the tumor volume at day 0.

IV. DISCUSSION

Cancer is a serious health problem in the world. According to a report by the Statics Korea, cancer is a leading cause of death in Korea and accounted for 28% of all deaths in 2012. There is no clear solution to conquer cancer; therefore, it's important to reduce cancer risk factors and to discover new chemopreventive/therapeutic agents. There might be many ways to minimize risk factors and to develop anti-cancer agent. The World Health Organization (WHO) and the National Cancer Institute (NCI) have established dietary guidelines to help people reduce the cancer risk. And, it's known that 60% of approved anti-cancer drugs are based on natural product [19].

In the present study, we investigated anti-cancer effects of 511 natural products. Notably, we demonstrated 14 natural products have growth inhibitory effects in premalignant, NSCLC cell lines with minimal effect on normal HBE cell. We selected one potent drug (compound **1**) out of 14 natural products and attempted to identify underlying mechanisms for the chemopreventive /therapeutic activities of the drug.

We found that compound **1** inhibits the viability and anchorage - independent colony formation of premalignant HBE and a subset of NSCLC cell lines by inducing apoptosis. We also observed that compound **1** suppressed the growth of NSCLCL xenograft tumor in mice.

In our effort to identify the mechanisms underlying chemopreventive/ therapeutic activities of compound **1**, we found that the drug has ability to

inhibit the IGF-IR signaling pathway. By the Receptor tyrosine kinase array, we demonstrated that pp70S6K is decreased by compound **1**. We found that the downstream of IGF-IR signaling pathways is involved. As it is generally accepted that the IGFs exhibits tumorigenic actions mainly through the IGF-IR, we searched IGF-1 and IGF-2 expression. And we discovered that compound **1** suppresses IGF-IR signaling pathway by regulating IGF2. Also, conditioned medium from compound **1** treated H1299 showed a significant reduction in IGF-IR signaling when compared with control.

Many studies have demonstrated that IGF-IR signaling is related with cell survival signal that protects against apoptosis by activating a number of downstream effectors including Akt, which plays a key role in cell cycle progression and angiogenesis stimulation. Furthermore, dysregulation of the IGF system is well recognized as a contributor to progression of cancer. Because IGF1 and IGF2 levels in bronchial tissue specimens containing high-grade dysplasia were significantly higher than in those containing normal epithelium [16]. So, targeting IGF axis as an anticancer therapeutic strategy is proper process.

In conclusion, our results provide *in vitro* and *in vivo* evidence for the first time that compound **1** has anti-tumor activities against lung cancer. It selectively blocks IGF-IR signaling pathway, leading to suppression of a major anti-apoptotic pathway. These results suggest that compound **1** is a potential anti-cancer chemopreventive/therapeutic agent.

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국문초록

암은 인간의 사망 원인 중 가장 큰 요인으로 알려져 있다. 그 중에서 폐암이 전체 암의 약 14%에 이르며, 5년 생존율은 20%미만에 불과한 것으로 집계되었다. 이에, 효과적인 항암치료법을 개발하기 위한 꾸준한 노력이 진행되어 왔으며 천연물이 항암제 신약의 원천물질로서 한 부분을 담당해오고 있다.

본 연구에서는 항암제 개발에 선도 물질로서 사용 가능성이 있을 것으로 사료되는 새로운 천연물질들을 찾기 위해 본 실험실에서 보유하고 있는 천연물 library를 screening 하였다. 폐암에서 약 80-90%가 비소세포 폐암(Non-small cell lung cancer)으로 가장 큰 부분을 차지 하고 있기에, 여러 비소세포 폐암과 정상세포를 대상으로 총 511개의 천연물 화합물을 screening 하였다. 정상세포에는 영향을 적게 미치고 비소세포 폐암에 더 특이적으로 효과를 나타내는 14개의 물질을 최종적으로 선도 물질로서 가능성이 있는 화합물로 선정할 수 있었다. 그리고 이 중에서 2가지 천연물 화합물을 폐암 세포 주에서의 심층적인 기전을 연구하기 위해 선택하였다. 우선 *in vitro* 상에서의 효과를 확인한 결과, 비소세포 폐암의 proliferation과 colony formation을 감소시켰으며 세포사멸을 유도하는 것으로 나타났다. 또한, 비소세포 폐암을 이용하여 xenograft tumor model을 확립하여 *in vivo* 상에서 그 효과를 확인한 결과, 약물처리군에서 tumor volume을 뚜렷하게 감소시켰다. 또한, 약물 처리 군에서 IGF-IR 신호 전달체계가 뚜렷하게 저하되는 것으로 나타나 후보물질의 항암활성을 매개하는 기전으로 추정된다. *In vitro, in vivo* 상에서의 연구결과를 종합할 때, 후보물질은 천연물 유래의 잠재적 신약후보 물질로 제시할 수 있을 것으로 기대된다.

주요어 : 폐암, 천연물 신약

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