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Original Article

β-sitosterol suppresses fibroblast growth factor and epidermal growth factor receptors to induce apoptosis and inhibit migration in lung cancer: an in vitro study

Shun-An Kan[1], Musarat Hussain[2], Chikondi Jassi[3], Wei-Wen Kuo[2,4,5], Chia-Hua Kuo[6,7], Pei-Ying Pai[8,9], Shu-Hui Lin[10,11,12], Yueh-Min Lin[13,14*], Chih-Yang Huang[3,15,16,17,18*], Shinn-Zong Lin[19,20*]

[1]Taipei Veterans General Hospital, Taipei 112, Taiwan; [2]Department of Biological Science and Technology, China Medical University, Taichung 40402, Taiwan; [3]Cardiovascular and Mitochondrial Related Diseases Research Center, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien 970, Taiwan; [4]Ph.D. Program for Biotechnology Industry, China Medical University, Taichung 40402, Taiwan; [5]School of Pharmacy, China Medical University, Taichung 40402, Taiwan; [6]Laboratory of Exercise Biochemistry, University of Taipei, Taipei 11153, Taiwan; [7]School of Physical Education and Sports Science, Soochow University, Suzhou 215021, Jiangsu, China; [8]School of Medicine, College of Medicine, China Medical University, Taichung 40402, Taiwan; [9]Division of Cardiovascular Medicine, Department of Medicine, China Medical University Hospital, Taichung 40447, Taiwan; [10]Department of Surgical Pathology, Changhua Christian Hospital, Changhua 50094, Taiwan; [11]Department of Post-Baccalaureate Medicine, College of Medicine, National Chung Hsing University, Taichung 400, Taiwan; [12]Department of Medical Laboratory Science and Biotechnology, Central Taiwan University of Science and Technology, Taichung 40601, Taiwan; [13]Department of Pathology, Changhua Christian Hospital, Changhua 500209, Taiwan; [14]School of Medicine, Chung Shan Medical University, Taichung 402, Taiwan; [15]Department of Medical Research, China Medical University Hospital, China Medical University, Taichung 40402, Taiwan; [16]Department of Biotechnology, Asia University, Taichung 413305, Taiwan; [17]Center of General Education, Buddhist Tzu Chi Medical Foundation, Tzu Chi University of Science and Technology, Hualien 970, Taiwan; [18]Graduate Institute of Biomedical Sciences, China Medical University, Taichung 404, Taiwan; [19]Bioinnovation Center, Buddhist Tzu Chi Medical Foundation, Hualien 970, Taiwan; [20]Department of Neurosurgery, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien 970, Taiwan. [*]Equal contributors.

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Abstract: β-Sitosterol (BS), is a significant bioactive component of phytosterols found in plants, foods, and dietary supplements. Its nutritional benefits include lowering of cholesterol levels, boost immune system as well as reduce inflammation. Previous studies have demonstrated its significant anticancer effects across various human cancers. However, the specific mechanisms of action of BS in lung cancer remain unclear. This study aimed to investigate the mechanisms through which BS exerts its anticancer properties in human lung cancer cells, focusing on its anti-proliferative, apoptotic, cytotoxic, and anti-migratory effects. We conducted an in vitro study to assess the effects of BS on lung cancer cell lines A549 and H1975. We used a range of assays, including MTT, western blot, wound healing, transwell migration, immunofluorescence, TUNEL, and cell survival assays, to evaluate the impact of BS on cell proliferation, apoptosis, cytotoxicity, and migration. Our findings indicate that BS inhibits the proliferation of lung cancer cells in a time- and dose-dependent manner. It significantly promotes apoptosis and impairs both cancer cell migration and survival. Additionally, BS suppresses the expression of both fibroblast growth factor receptor-1 (FGFR1) and epidermal growth factor (EGFR), leading to the downregulation of the PI3K/AKT/mTOR/CD1 signaling pathway. BS demonstrates significant anticancer potential in lung cancer cells by inhibiting proliferation, inducing apoptosis, and reducing cell migration. These effects are likely mediated by the concurrent downregulation of FGFR1 and EGFR, leading to the inhibition of the PI3K/AKT/mTOR/CD1 signaling pathway, thereby warranting further investigation of BS as a potential therapeutic agent for lung cancer.

Keywords: Apoptosis, epidermal growth factor receptor, fibroblast growth factor receptor 1, lung cancer, β-Sitosterol (BS)

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Introduction

β-Sitosterol (BS) is a prominent bioactive compound widely distributed across various phytosterols [1]. Phytosterols are plant-derived molecules structurally similar to cholesterol, with differences primarily in their side chains [2]. Found abundantly in foods such as nuts, vegetable oils, seeds, legumes, and fruits, BS has been extensively studied for their nutritional benefits, which include lowering cholesterol levels, boosting the immune system, and reducing inflammation, among others [1-3]. Beyond these well-documented advantages, both in vitro and in vivo studies have demonstrated that β-Sitosterol possesses significant anticancer effects across various cancer types [4].

In the context of cancer, Lung cancer is the second leading cause of death globally. According to the World Health Organization (WHO) and Global cancer (GLOBOCAN) reports, cancer accounted for, close to 10 million deaths globally in 2022 [3-5]. Lung cancer is a major cause of cancer-related deaths with an estimated 18.7% of all cancers globally by 2022 [5, 6]. This is significant because the death rate from lung cancer is almost equal to the number of cases, and the 5-year survival rate is still incredibly low at overall 22% [7-9]. Lung cancer is mostly treated with radiation, chemotherapy, immunotherapy, and surgery [10]. However, most of these strategies have met challenges inform of resistance, unwanted toxicities, eventual tumor recurrences and consequently deaths. Therefore, investigating for novel compounds with both nutritional and potential anticancer effectiveness, notable safety, and low toxicity in the battle against lung cancer has become imperative.

In both preclinical and clinical studies, phytochemicals extracted from plants have demonstrated potential as medicinal sources [11]. According to the findings, the cytotoxic analysis of plant extracts or isolated compounds has made it feasible for treatments that specifically target rapidly reproducing cancer cells. Several compound of nutritional value and potent anticancer activities, such as β-Sitosterol (BS), can be extracted from plants [12]. Previous studies have demonstrated the potential protective effects of phytosterols and their derivatives against a variety of chronic illnesses, such as inflammation, arteriosclerosis, cancer, and ulcers [13]. Numerous pharmacological characteristics of BS have been demonstrated through experimentation, including its anti-diabetic effects, hypolipidemic effects on hyperlipidemia, hepatoprotective, anti-atherosclerosis, and protection against immune modulation-damage by oxidative stress [14]. BS's biodegradability ensures the safety of the drug delivery system [15]. Because of its safety, low toxicity, and effectiveness, BS has recently been investigated for different diseases.

Fibroblast growth factor receptor 1 (FGFR1) is a receptor tyrosine kinase that regulate a wide range of cellular processes, such as metabolism, migration, survival, proliferation, and differentiation [16]. Cancers have been linked to FGFR1 amplifications, translocations, and point mutations [17]. Mutations in FGFR1 that are aberrant in their development have been connected to a variety of cancer types [18]. PI3K/AKT/mTOR, signal transducer and activator of transcription (STAT), and RAS-RAF-MAPK are the significant downstream pathways activated when the fibroblast growth factor receptor is activated. Activation of FGFR1 promotes adherence of docking proteins and initiates downstream signaling cascades [19]. For instance, FGFR1 activation triggers the activation of PI3K/AKT pathway which consequently activates and regulate several other cellular processes [20]. Cell division, proliferation, and survival are just a few of the biological processes that are regulated by the signaling cascade activated by FGFR1 [21]. Therefore, our aim was to investigate the role of phytosterol, BS in lung cancer in the context of FGFR1. Our study focused on β-sitosterol (BS) and FGFR1 due to BS's previously demonstrated safety, low toxicity, and effectiveness in various cancers, as well as the consistent association of FGFR1 with cancer aggressiveness.

Materials and methods

Chemicals and reagent

β-Sitosterol was purchased from Cayman Chemicals (Ann Arbor, MI, USA). The apoptosis detection kit (V13242) was obtained from Thermo Fisher Scientific (USA). Antibodies against AKT, phosphorylated AKT, and phosphorylated PI3K were procured from Cell Signaling Technology (Danvers, MA, USA). Antibodies against β-actin, caspase-3, Bcl-2,

Bax, and cyclin D were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Antibodies against PARP were sourced from Cell Signaling Technology (Danvers, MA, USA). Antibodies against FGFR1 (AF6156) were purchased from Affinity Biosciences. Phosphorylated FGFR1 (#52928s), mTOR (#2983), and phosphorylated mTOR (#2974) antibodies were acquired from Cell Signaling Technology. Additionally, Vimentin (sc-32322) and E-cadherin (sc-8426) antibodies were obtained from Santa Cruz Biotechnology. FGFR1 activator, SUN11-602 was purchased from MedChem Express (Cat# HY-101493). Both Gefitinib and Nintendanib were purchased from MedChem Express (Cat# HY-50895 and HY-50904 respectively).

Cell culture

Cancer cell lines A549, H1975, HA22T were acquired from the Cancer Research Institute in Taichung, Taiwan. These cells were maintained in DMEM high glucose medium, supplemented with 10% (v/v) inactivated heat-FBS (Invitrogen), 10% penicillin and streptomycin, incubated in a humidified atmosphere of 5% CO_2 temperature of 37°C. The medium was changed every three days.

Cell viability- MTT

Cells were seeded at 5×10^5 cell/plate in 6-well plates then incubated at various times with varying concentrations of BS (25, 50, 100, 200, and 400 μM). After discarding the medium, it was replaced with a freshly made medium with 5 mg/ml MTT solution then incubated for three hours. Precipitated crystals were then dissolved by DMSO. This solution was transferred to 96 well plate at 200 uL per well. The OD values were then read using microplate reader (Biotech) and/or the SpectraMax M3 (Molecular Devices) with Sofmax software Pro V5 5.4.1 at 570 nm absorbance.

Extraction of cellular protein and Western blot analysis

Cells were plated in 10 cm dishes at 1×10^6 cells per plate. After the appropriate treatment and incubation time, cells were washed with PBS and lysed using RIPA buffer. The lysate was collected in clean, sterilized tubes. Total protein was extracted by centrifuging the lysate at 12,000 g for 30 minutes at 4°C. Protein concentration was determined using the Bio-Rad Protein Quantification Protocol. For western blot analysis, 30-40 μg of protein per well was loaded for separation by molecular weight using SDS-PAGE (7%, 8%, 10%, 13%, and 15% gels). The separated proteins were transferred onto PVDF membranes (Millipore, Bedford, MA; pore size 0.45 μm). Nonspecific binding sites were blocked using 5% non-fat milk in TBST (TBS with 1% Tween 20), followed by overnight incubation at 4°C with the appropriate primary antibodies. After washing with TBST, the membranes were incubated with the relevant secondary antibodies for 1 hour. Following further washes with TBST, protein expression was visualized using HRP chemiluminescence (ECL) and analyzed with ImageQuant software.

TUNEL assay

The effect of β-sitosterol (BS) on the induction of apoptosis was evaluated using the TUNEL assay. Cells were seeded in 4-well plates at a density of 2×10^4 cells per well and incubated for 24 hours before being treated with various concentrations of BS for an additional 48 hours. After 48 hours of treatment, the cells were washed with PBS and fixed with 4% paraformaldehyde (PFA). The cells were then permeabilized using 0.1% Triton X-100 solution. Following another PBS wash, the cells were stained using the In-Situ Cell Death Detection Kit (Sigma-Aldrich) for one hour, followed by DAPI staining to label the nuclei. Apoptotic cells were visualized and quantified using an Olympus DP73 fluorescence microscope (Tokyo, Japan).

Wound healing assay

Cells were seeded in 6-well plates at a density of 3×10^5 cells per well and incubated for 24 hours. β-Sitosterol (BS) was administered at concentrations of 0, 25, 50, 100, 200, and 400 μM for 12, 24, and 48 hours. A scratch wound was created using a pipette tip, and wound closure was monitored with images captured at various time points.

Transwell assays

Cells were seeded at 5×10^3 in 1000 μL of DMEM into each transwell chamber with an

8-μm pore-sized filter membrane (Millipore, Schwalbach, Germany). Fresh media containing 10% FBS was added to the lower chamber, while cells were seeded in media without FBS in the upper chamber. The cells were incubated at 37°C with 5% CO_2 to allow the migration through the transwell inserts. After 24 hours of incubation, non-migrated cells were removed using cotton swabs, and migrated cells on the underside of the membrane were fixed and stained with methanol and 0.2% crystal violet respectively at room temperature. Migrated cells were then checked in five randomly selected fields at 10× magnification using an Olympus inverted microscope and pictures taken.

Immunofluorescence assay

Immunofluorescence (IF) assay was employed to assess FGFR1 expression in A549 cells. A549 cells were seeded on 4-well slides and treated with varying doses of β-sitosterol (BS) for 24 hours. Following treatment, the cells were washed three times with PBS. To fix the cells on coverslips, 4% paraformaldehyde (PFA; Thermo Fisher Scientific, Waltham, MA, USA) was applied for 30 minutes at room temperature. Permeabilization was carried out using 0.1% Triton X-100 in PBS for 10 minutes. The cells were then blocked with blocking buffer (Thermo Fisher Scientific, Waltham, MA, USA) for one hour. This was followed by overnight incubation with primary antibodies, and subsequent staining with Alexa Fluor 488-conjugated anti-FGFR antibodies, diluted in 1% FBS in PBST (Thermo Fisher Scientific, Waltham, MA, USA). IF images were captured using an immunofluorescence microscope.

Statistical analysis

Graph Pad Prism 5 (Graph Pad Software) was used for statistical analysis. The mean ± SD of at least three independent experiments was used to present the representative results. Data were analyzed using one-way or two-way ANOVA, depending on the number of groups and the comparisons being made. *P*-values below 0.05 were considered statistically significant.

Results

β-sitosterol reduced cell viability in multiple cancer cell lines

Firstly, we aimed to investigate the effects of beta-sitosterol (BS) on the viability and proliferation of A549, a non-small cell lung cancer (NSCLC) cell line. To assess this, A549 cells were treated with different concentrations of BS, and an MTT assay was performed to evaluate cell viability. Treatment with BS at concentrations of 25, 50, 100, 200, and 400 μM resulted in a dose- and time-dependent reduction in cell viability. At 24, 48, and 72 hours, BS exhibited potential anti-cancer effects with half-maximal inhibitory concentrations (IC50) of 165.3, 53.2, and 33.4 μM, respectively (**Figure 1A-C**).

Next, we sought to evaluate the effects of BS on another lung cancer cell line, H1975, which is resistant to tyrosine kinase inhibitors, as well as on HA22T (hepatocarcinoma) and LoVo (colorectal cancer) cell lines. After 48 hours of treatment, BS reduced cell viability in all tested cell lines, with IC50 values of 355.3, 431.8, and 267.1 μM, respectively (**Figure 1D-F**).

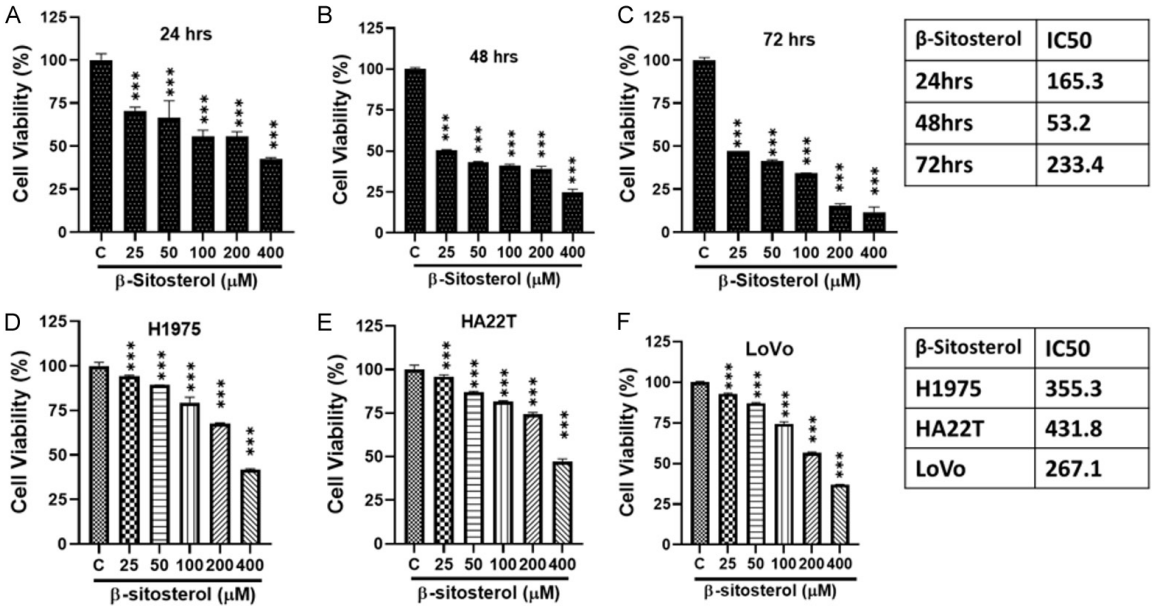
These results preliminarily suggest that BS possesses broad-spectrum anti-cancer properties, demonstrating efficacy not only against lung cancer but also against hepatocarcinoma and colorectal cancer.

β-sitosterol induced apoptosis in A549 none-small cell lung cancer cell lines

Secondly, we investigated the effect of BS on apoptosis in lung cancer cells. A549 cells were treated with varying concentrations of BS, followed by protein extraction for western blot analysis to assess the expression of apoptotic markers, including PARP1, cleaved caspase-3, Bcl-2, and Bax. The results demonstrated a dose-dependent upregulation of PARP1, cleaved caspase-3, and Bax, alongside a downregulation of Bcl-2 (**Figure 2A**). To further confirm these findings, a TUNEL assay was conducted, revealing an increase in the number of TUNEL-positive cells, which also correlated with increase in BS concentrations, indicating enhanced apoptosis (**Figure 2B**).

β-sitosterol inhibited cell migration in A549 NSCLC none-small cell lung cancer and other cancer cell lines

Lung cancer aggressiveness is characterized by its invasiveness and ability to migrate to other tissues. We aimed to investigate whether BS has the potential to inhibit cell migration. To achieve this, we performed wound healing and transwell cell migration assays on A549 cells,



β-Sitosterol	IC50
24hrs	165.3
48hrs	53.2
72hrs	233.4

β-Sitosterol	IC50
H1975	355.3
HA22T	431.8
LoVo	267.1

Figure 1. β-Sitosterol reduced viability in various cancer cell lines. Different cancer cell lines were treated with varying concentrations of β-Sitosterol (BS), and cell viability was assessed at different time points. Viability of the A549 (NSCLC) cell line at 24, 48, and 72 hours (A-C). Viability of the H1975 (lung cancer), HA22T (hepatocellular carcinoma), and LoVo (colorectal cancer) cell lines at 48 hours (D-F). Data are presented as the mean ± SD from three independent experiments. Statistical significance is indicated as *P ≥ 0.05, **P < 0.01, and ***P < 0.001. BS: β-Sitosterol, IC50: Half-maximal inhibitory concentration.

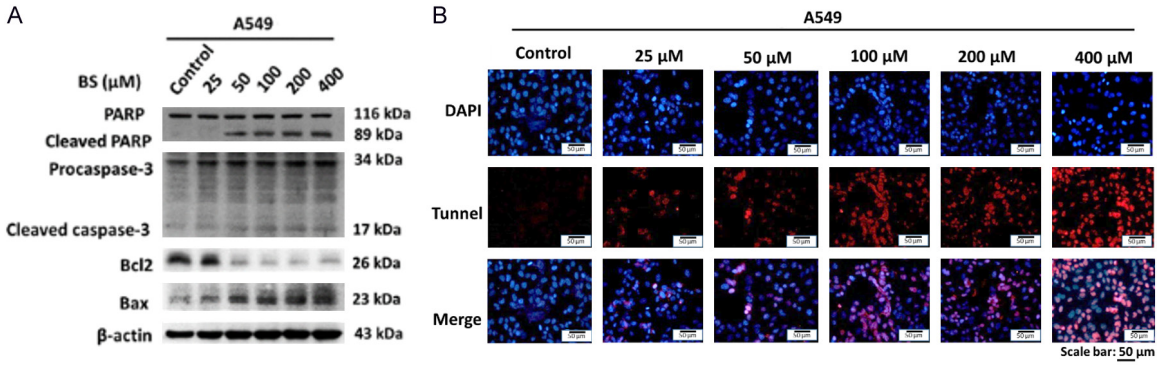


Figure 2. β-Sitosterol induced apoptosis in A549 cells lines. A549 lung cancer cell lines were treated with different concentrations of BS. Cell response to treatment in terms of apoptosis was determined by both western blot (A) and Tunel apoptotic assay (B).

using a range of BS treatments. The results demonstrated a BS dose-dependent inhibition of cell migration in both assays, as indicated by reduced cell gap filling (**Figure 3A**) and a decreased number of cells migrating through the membrane (**Figure 3B**).

Since the transition from an epithelial to a mesenchymal phenotype (EMT) is associated with increased migratory potential, we conducted Western blot analysis on protein extracts from these treated cells to assess the expression of EMT markers, E-cadherin and vimentin. The results showed that E-cadherin expression increased with increasing BS dose, while vimentin expression decreased, suggesting a transition from a mesenchymal to an epithelial phenotype (MET) (**Figure 3C**). Additionally, we investigated the ability of BS to inhibit cell migration in other cell lines, including H1975, HA22T, and LoVo, using the wound healing assay at a 48-hour time point. Consistently, BS

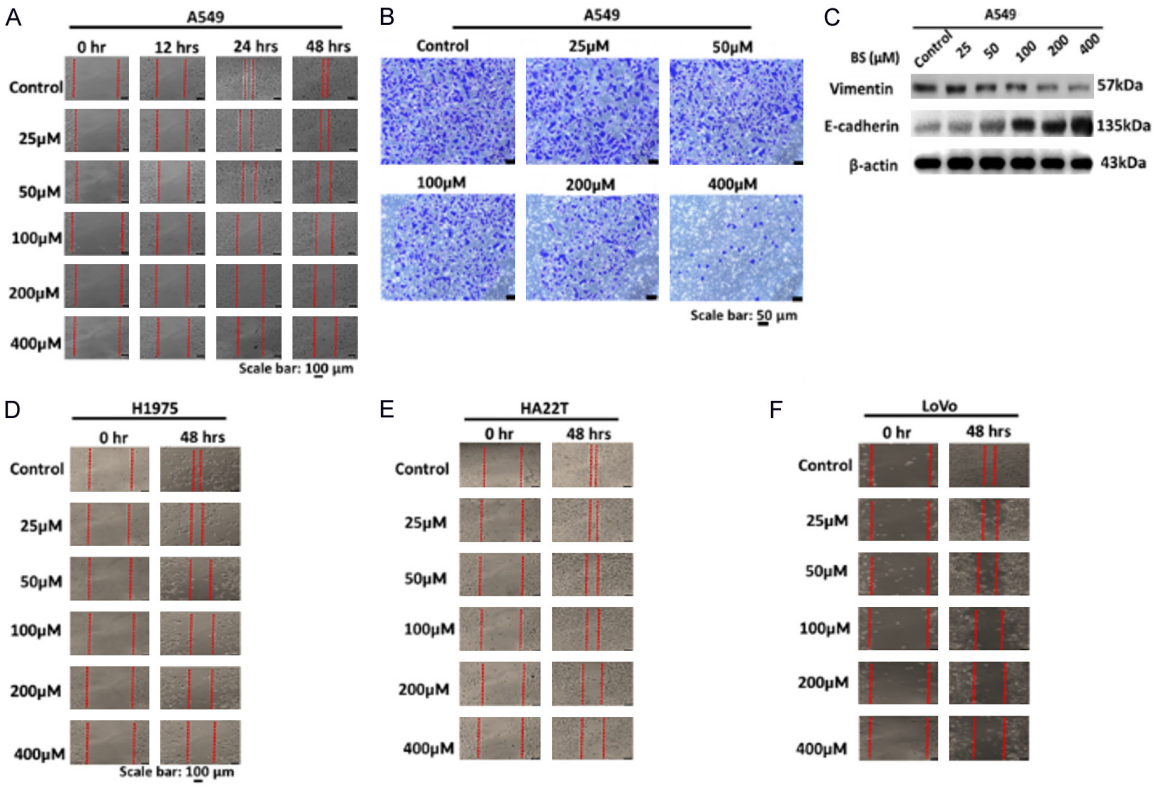


Figure 3. β-Sitosterol inhibited cell migration in various cancer cell lines. A549 lung cancer cell lines were treated with different concentrations of BS. Cell response to treatment in terms of migration ability was determined using wound healing and transwell assay (A, B). Expression of two EMT markers in response the treatment was determined by western blot (C). Other cell lines, for lung, hepatocellular carcinoma and colon cancer, H1975, HA22T and LoVo respectively were assesses for their migration ability at 48 hours in response to the treatment (D-F).

inhibited cell migration in all cell lines in a dose-dependent manner (**Figure 3D-F**). These results suggest that BS has the potential to inhibit cell migration in lung cancer cells as well as in hepatocellular carcinoma and colorectal cancer cells.

β-sitosterol suppressed FGFR1 and downstream PI3K/AKT/Cyclin D1 signaling in A549 cells

Activation of the fibroblast growth factor receptor 1 (FGFR1), a receptor tyrosine kinase, has been implicated in the aggressiveness and drug resistance of lung cancer. Downregulation or therapeutic targeting of FGFR1 has been shown to improve cancer treatment outcomes and patient survival. This study aimed to investigate whether BS can downregulate FGFR1 in lung cancer cells. To address this, A549 lung cancer cells were treated with different concentrations of BS, then FGFR1 expression was assessed by western blot and immunofluorescence. As shown in **Figure 4A**, BS treatment reduced the expression of FGFR1, particularly its phosphorylated form, in a dose-dependent manner. Immunofluorescence analysis further confirmed this reduction at concentrations of 25 and 400 μM (**Figure 4B**).

Given that the PI3K/AKT/mTOR is one of the pathways regulated by FGFR1, we also examined its response to BS treatment. Western blot analysis revealed a dose-dependent downregulation of the PI3K/AKT/mTOR pathway and Cyclin D1, a key regulator of cell cycle progression (**Figure 4C**). Since activation of this pathway is known to promote cancer cell survival, we conducted a cell survival assay over three weeks. BS-treated cells demonstrated significantly reduced survival compared to the untreated control, with a dose-dependent effect observed (**Figure 4D**).

To further investigate the downregulation of FGFR1 across different cancer types, we treated H1975 (lung cancer), HA22T (hepatocellular carcinoma), and LoVo (colorectal cancer) cell

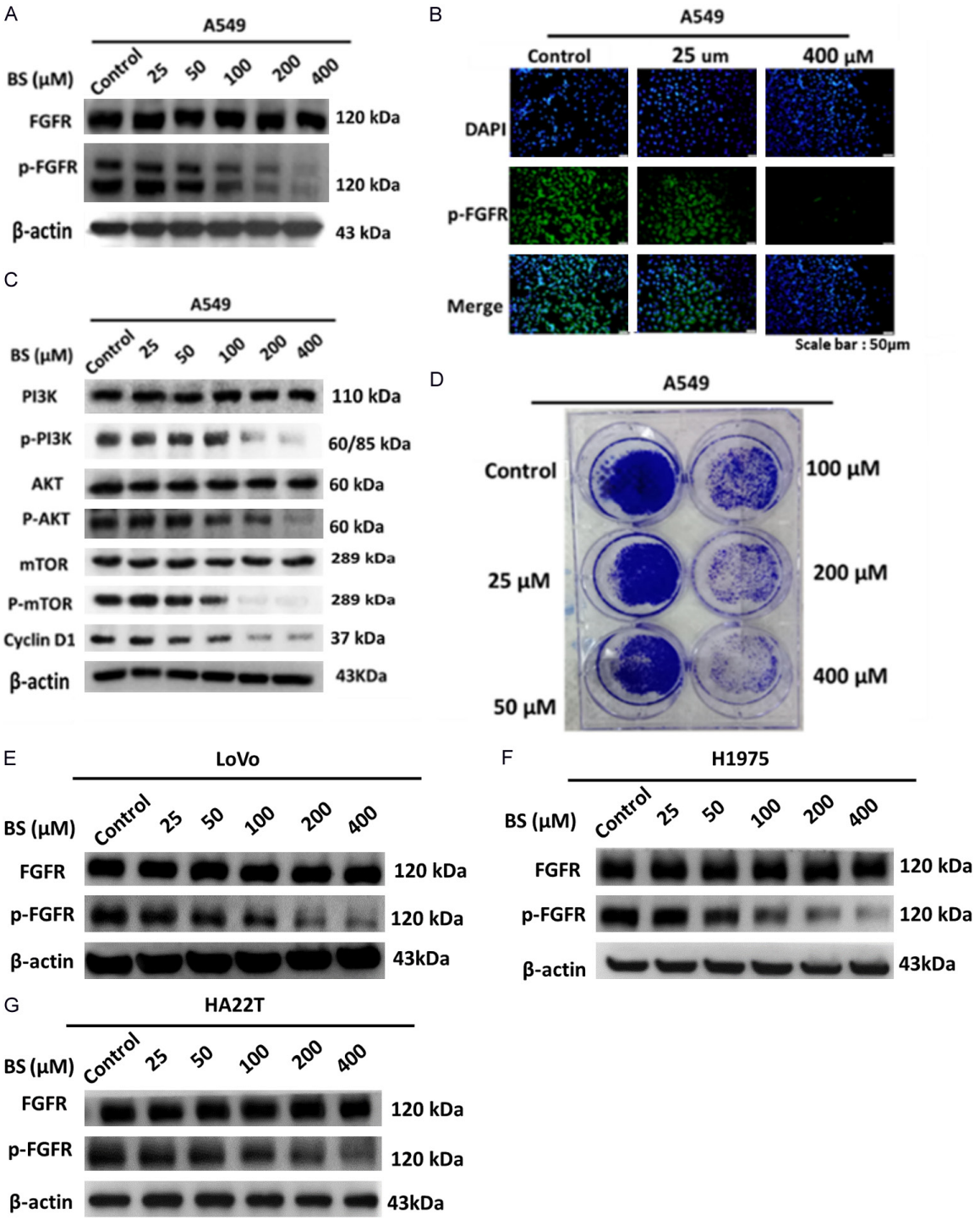


Figure 4. β-Sitosterol downregulated FGFR1 and PI3K/AKT/mTOR/Cyclin D1 pathway. Following treatment with BS, protein was extracted from A549 cells, to determine the expression of FGFR1 using western blot (A). The cells were also subjected to immunofluorescence to determine the expression FGFR1 (B). Furthermore, PI3K/AKT/mTOR/CyclinD1 pathway was determined using western blot (C). Additionally, survival rate of the cells after treatment was also determined (D). We then determined the effect of β-Sitosterol on the expression of FGFR1 in three other cell lines for colorectal, lung and hepatocellular carcinoma (E-G).

lines with BS. In all cases, a dose-dependent downregulation of FGFR1 was observed, confirming the broad-spectrum anti-cancer effect of BS (**Figure 4E-G**).

These findings suggest that BS has the potential to downregulate FGFR1, thereby inhibiting the PI3K/AKT/mTOR pathway and potentially other related pathways, ultimately reducing cancer cell survival across multiple cell types.

B-sitosterol suppressed exogenously activated fibroblast and epidermal growth factor receptors (FGFR1/EGFR) and the downstream PI3K/AKT/Cyclin D1 pathway in A549 cells

In section 3.4, we demonstrated that BS downregulated the endogenous FGFR1 and the downstream PI3K/AKT/mTOR/Cyclin D1 pathway. Numerous studies have shown that FGFR1 activation contributes to tumor cell aggressiveness and survival by engaging various signaling pathways. Our aim was to exogenously activate FGFR1 in A549 cells and assess its impact on downstream survival pathways and related biological processes. Additionally, we sought to determine whether BS could reverse these processes.

First, A549 cells were treated with different concentrations of the FGFR activator, SUN11602 to establish the optimal concentration for subsequent experiments. Western blot analysis revealed a dose-dependent upregulation of FGFR 1 with increasing concentrations of SUN11602 (**Figure 5A**), leading us to select 30 μM for further experiments.

Next, cells were treated with FGFR activator, BS, and their combination, alongside a control group. Western blot analysis showed that BS alone reduced FGFR1 expression, whereas the activator increased FGFR1 levels. Notably, co-treatment with BS and the activator reversed FGFR1 activation, as evidenced by a reduction in FGFR1 expression compared to activation by SUN11602 alone (**Figure 5B**). This finding was corroborated by immunofluorescence analysis, which demonstrated similar FGFR1 expression patterns (**Figure 5C**).

We then assessed the impact on the PI3K/AKT/mTOR/Cyclin D1 pathway. BS treatment downregulated this pathway, while FGFR activation upregulated it. Co-treatment with BS and the activator reversed this upregulation (**Figure 5D**). To explore whether these effects extended to apoptosis, western blot analysis was performed, showing that BS induced apoptosis, whereas the activator suppressed it. However, co-treatment enhanced apoptosis, as indicated by increased cleaved PARP, cleaved caspase-3, Bax, and decreased Bcl-2 levels (**Figure 5E**).

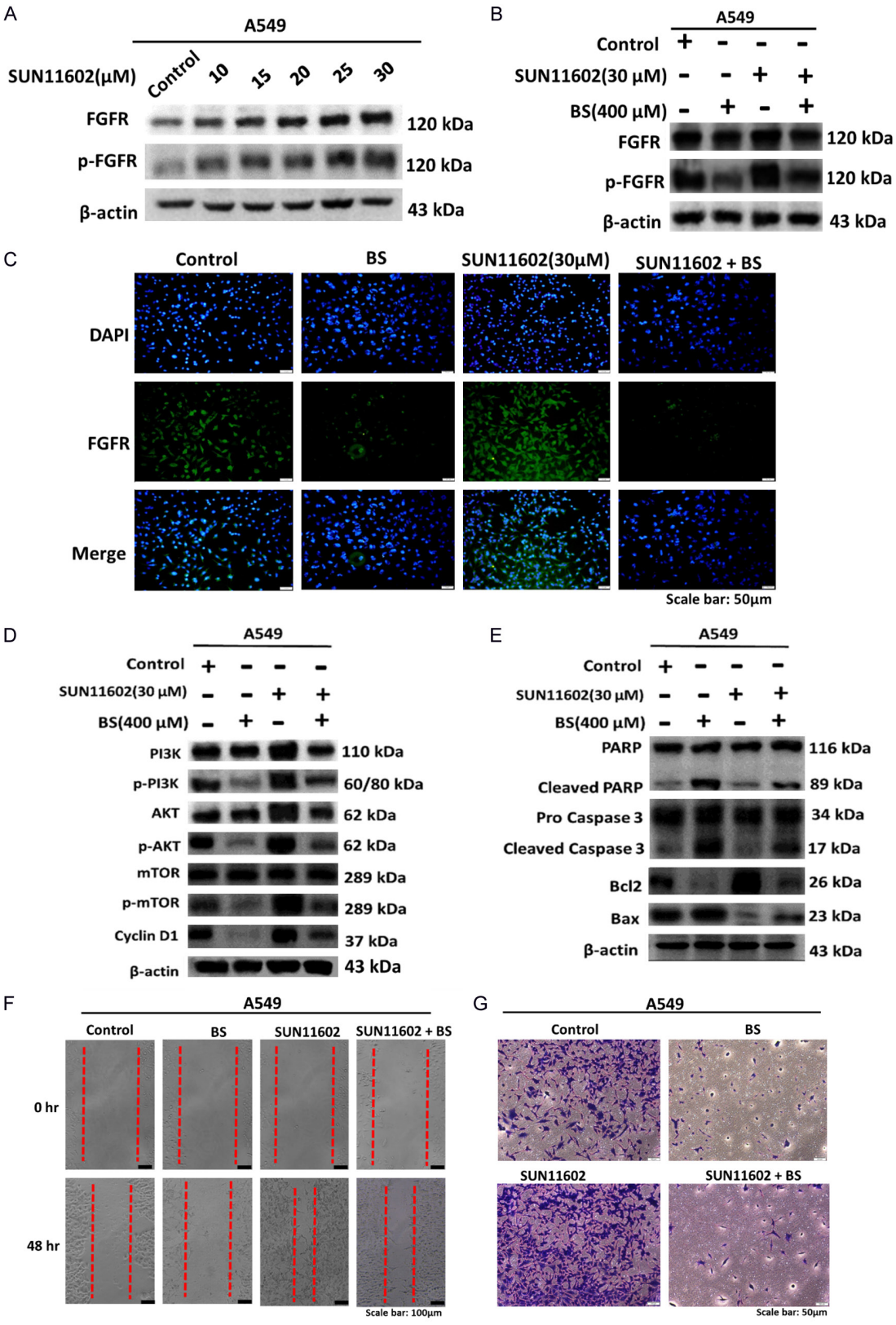
Since BS had previously shown effects on cell migration (Section 3.3), we investigated this in the context of FGFR1 activation. Wound healing and transwell migration assays revealed that BS inhibited cell migration, while the FGFR activator enhanced it. Co-treatment with BS reversed the activator-induced migration (**Figure 5F**, **5G**). Furthermore, western blot analysis of epithelial-mesenchymal transition (EMT) markers showed that the activator-induced upregulation of Vimentin and downregulation of E-cadherin were reversed by BS co-treatment (**Figure 5H**).

Studies have shown that β-sitosterol downregulate EGFR [15, 22, 23], another tyrosine kinase receptor implicated in carcinogenesis and cancer progression. Since EGFR activation stimulates the PI3K/AKT pathway, we aimed to investigate whether the observed antitumor effects of BS involve the co-inhibition of both FGFR1 and EGFR. For this purpose, we treated A549 cells with nintedanib, an FGFR inhibitor, alone and in combination with BS to evaluate its effect on both receptors. The results indicated that FGFR1 inhibition did not affect β-sitosterol's ability to suppress the PI3K/AKT pathway. However, the combination of β-sitosterol and nintedanib enhanced pathway suppression compared to either treatment alone, suggesting the involvement of an alternative mechanism (**Figure 5I-K**). EGFR inhibition did not alter β-sitosterol's suppression of the PI3K/AKT pathway, and the combination of gefitinib with β-sitosterol enhanced the effect (**Figure 5L-N**). Given our previous findings that β-sitosterol reverses FGFR1 activation-induced PI3K/AKT signaling (via SUN1102), these results suggest that the observed anticancer effects may involve the concurrent downregulation of FGFR1 and EGFR, ultimately suppressing the PI3K/AKT/mTOR/Cyclin D1 pathway.

These results suggest that BS has potential as an adjunct therapy, improving treatment efficacy in scenarios where FGFR1 and EGFR mediates lung cancer survival and progression.

β-sitosterol did not exhibit cytotoxicity to normal lung cells

The efficacy of cancer treatment relies on selectively targeting cancer cells while minimiz-


A
A549
SUN11602(μM)
Control
10
15
20
25
30
FGFR
120 kDa
p-FGFR
120 kDa
β-actin
43 kDa
B
A549
Control
SUN11602(30 μM)
BS(400 μM)
FGFR
120 kDa
p-FGFR
120 kDa
β-actin
43 kDa
C
Control
BS
SUN11602(30μM)
SUN11602 + BS
DAPI
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Scale bar: 50μm
D
A549
Control
SUN11602(30 μM)
BS(400 μM)
PI3K
110 kDa
p-PI3K
60/80 kDa
AKT
62 kDa
p-AKT
62 kDa
mTOR
289 kDa
p-mTOR
289 kDa
Cyclin D1
37 kDa
β-actin
43 kDa
E
A549
Control
SUN11602(30 μM)
BS(400 μM)
PARP
116 kDa
Cleaved PARP
89 kDa
Pro Caspase 3
34 kDa
Cleaved Caspase 3
17 kDa
Bcl2
26 kDa
Bax
23 kDa
β-actin
43 kDa
F
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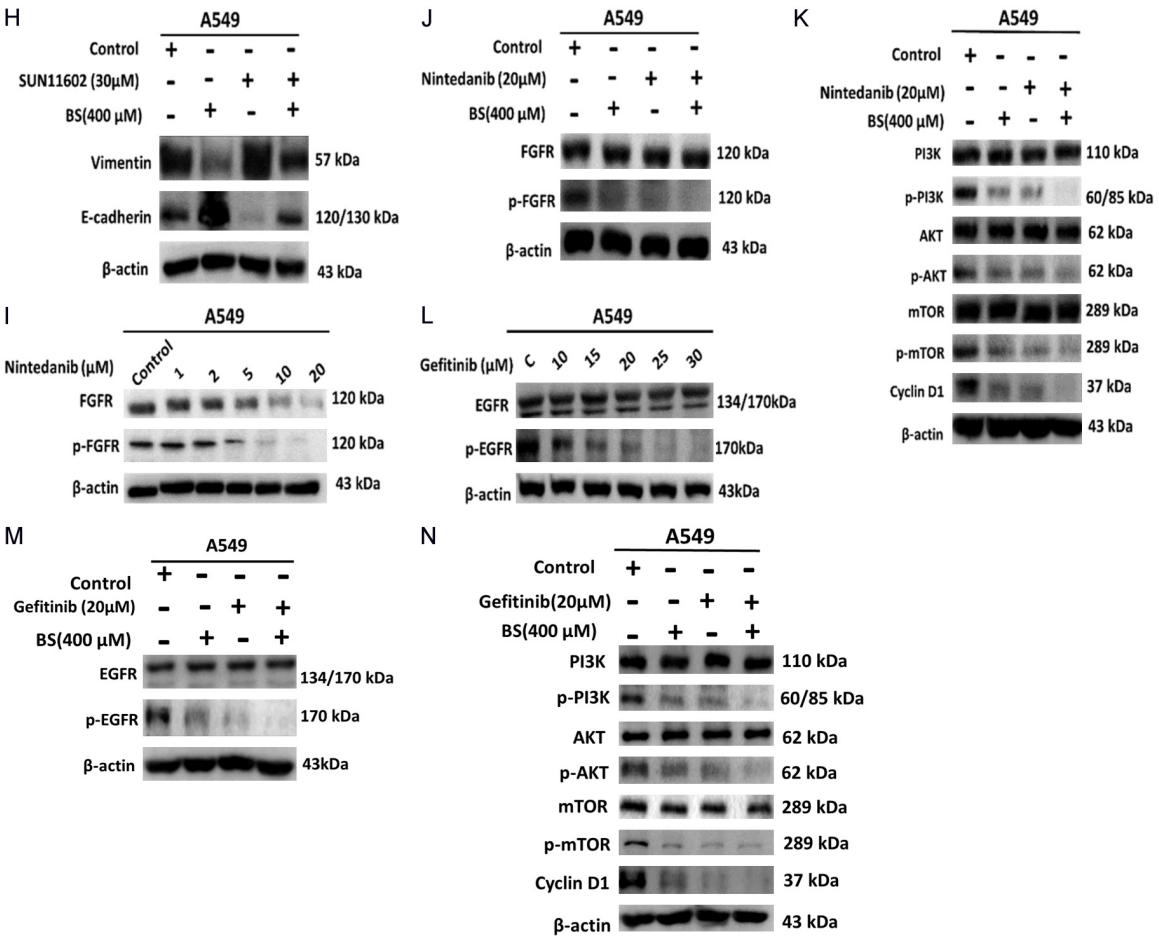

Figure 5. β-Sitosterol suppressed both FGFR and EGFR expression in A549 cells. A549 cells were subjected to FGFR activation by treatment with SUN11602. Activation efficiency were determined by western blot (A). The reversal of FGFR-activation by BS was determined by western blot and immunofluorescence (B, C). FGFR-mediated activation of PI3K/AKT/Cyclin D1 pathway was reversed by BS (D). The suppression of apoptosis by the activation of FGFR1 was reversed by BS (E). Cell migration mediated by the activation of FGFR was reversed by BS (F, G). Transition from epithelial to mesenchymal phenotype mediated by FGFR activation was reversed by BS (H). BS enhanced the effect of FGFR inhibition (I-K). BS enhanced the effect of EGFR inhibition (L-N).

ing toxicity to normal cells. However, many anticancer agents exhibit low selectivity, potentially causing harm to healthy tissues. To assess this aspect in β-sitosterol, we treated normal human lung epithelial cells (Beas-2B) with the same concentrations used in previous experiments and evaluated cell viability at 24, 48, and 72 hours. The results showed that β-sitosterol did not induce significant cytotoxicity in these cells (**Figure 6A-C**), suggesting its potential safety in minimizing off-target toxicity during treatment.

Discussion

While the nutritional benefits of various compounds for overall health are widely acknowledged, the exploration of novel compounds that offer both nutritional value and therapeutic potential is equally crucial. The search for innovative therapeutic strategies against cancer remains a pressing endeavor, with numerous plant-derived compounds demonstrating significant anti-cancer properties [24]. In this study we focused on β-sitosterol (BS), a bioactive phytosterol, naturally present in various plant-derived foods [25]. Based on previous studies, BS has been shown to possess various biological actions such as antioxidant, anti-diabetic, antimicrobial, immunomodulatory and anticancer activities [1, 14]. We aimed to investigate the in vitro anticancer effects of BS in lung cancer particularly FGFR1. Alterations of

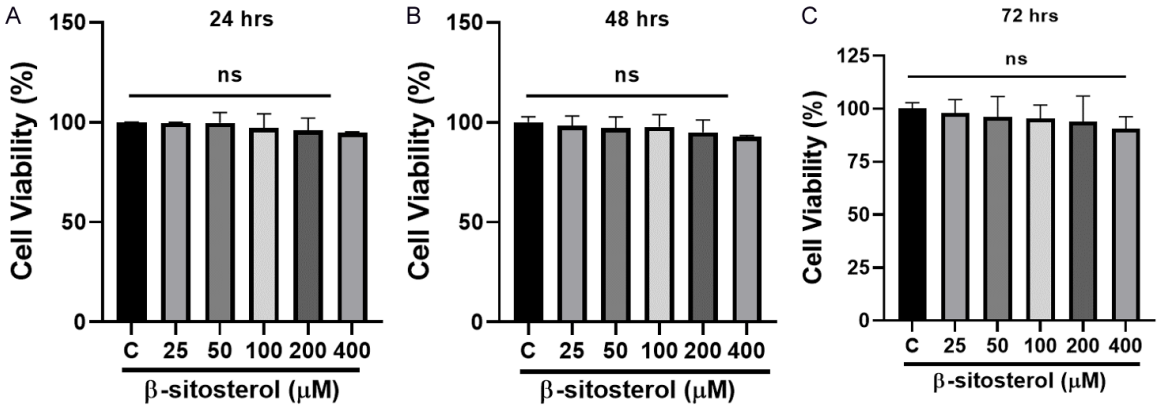

Figure 6. β-Sitosterol did not exhibit toxicity to normal cells. MTT assay was used to evaluate the effect of BS treatment in normal lung cells, Beas-2B on cell viability. No significant toxicity was observed at 24, 48 and 72 hours (A-C).

FGFR1 and EGFR have been found in various cancers [26, 27] and are said to mediate key compensatory bypass mechanisms to drug resistance [28]. Therefore, taking advantage of the beneficial effects of BS and explore experimentally against unwanted effects of these receptors becomes imperative. We have revealed that treating lung cancer cells with BS reduces cell viability. It is interesting to note that this effect was not only observed in lung cancer cells but also in hepatocellular carcinoma and colorectal cancer cells. This property is advantageous considering the existence of multiple cancers that all contribute to total number of mortalities. Many of the current drugs with multiple therapeutic functions have been experimented on and some are already used in the clinics [29]. Of note is the drug Paclitaxel, one of the drugs used in treatment of various cancers, thanks to its multiple targeting, first isolated from pacific yew tree [30]. Another property we have revealed about BS is the induction of apoptosis. Apoptosis is a cell programmed cell death, plays a role of balancing cell death and division in preventing uncontrolled cell proliferation and is useful in the treatment of cancers [31]. Previous studies have demonstrated that BS induces apoptosis in other cancer cells. Mary J. Ditty and Devaraj Ezhilarasan [29] have demonstrated that BS induced apoptosis in HepG2 cells by accumulating intracellular reactive oxygen species (ROS). Consistently our results demonstrated an induction of apoptosis in A549 cells and demonstrated the reduction of cell viability including HA22T hepatocellular carcinoma and Lovo colorectal cancer cells. Cell migration is one of the characteristics of aggressive cells invading into near or distant tissues [32]. Our study demonstrated that treatment with BS reduces the migration ability of various cancer cells and reduced cell survival in A549 cells in dose-dependent manner. These results are in line with Wang et al. [33] who demonstrated that Aloin, another phytocompound isolated from Aloe Vera species, inhibited the proliferation and migration of gastric cancer cells by regulating NOX2-ROS mediated pro-survival signaling pathway. Further to our findings, we revealed that BS downregulated FGFR1 in A549, both endogenous and the exogenously activated. FGFR1 has been implicated in various cancers to mediate cell survival, migration and cancer progression [34]. Owing to the biological processes that are mediated by FGR1 activation, most of which contribute to failure in treatment, the inhibition of these processes potentiates BS as lung cancer treatment that require further investigation. Furthermore, while validating FGFR1 inhibition by β-sitosterol, we confirmed its previously observed ability to inhibit EGFR, revealing the concurrent suppression of both receptors. This finding is significant, as FGFR1 and EGFR are both key drivers of cancer progression [19, 26, 27]. Our investigation of BS in lung cancer has been conducted in vitro, which warrants further investigation in vivo. Furthermore, due to frequency of resistant phenotypes, investigating the effects of β-sitosterol in resistant cell lines may provide valuable insights into its underlying mechanisms of action.

Conclusion

Our results have demonstrated that BS can reduce cell viability, induces apoptosis and inhibit cell migration in vitro. Although further investigation is required, the concurrent down-regulation of FGR1 and EGFR by BS might suggestively reveal the mechanism through which BS induce its anticancer effect. Collectively, these results suggest that in addition to its nutritional value, BS is a potential anticancer compound, not only in lung but also in other cancers.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yueh-Min Lin, Department of Pathology, Changhua Christian Hospital, No. 135, Nanxiao Street, Changhua 500209, Taiwan. Tel: +886-04-7238595; E-mail: 93668@cch.org.tw; Dr. Chih-Yang Huang, Cardiovascular and Mitochondrial Related Diseases Research Center, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, No. 707, Section 3, Central Road, Hualien 970, Taiwan. Tel: +886-4-22053366 Ext. 3313; Fax: +886-4-22032295; E-mail: cyhuang@mail.cmu.edu.tw; Shinn-Zong Lin, Bioinnovation Center, Buddhist Tzu Chi Medical Foundation, No. 707, Section 3, Central Road, Hualien 970, Taiwan. Tel: +886-4-38561825 Ext. 13201; Fax: +886-4-38562019; E-mail: Shinn-Zong@tzuchi.com.tw

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