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DB3 from Antarctic lichen inhibits the growth of B16F10 melanoma cells in vitro and in vivo

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Abstract

Malignant melanoma is a fatal disease with an increasing global incidence. Despite numerous studies focused on anti-cancer drugs, a variety of side effects of cancer treatment remain challenging. Thus, there is a pressing need to identify novel anti-cancer agents with minimal cytotoxicity and side effects. DB3 (1,3,7,9-tetrahydroxy-2,8-dimethyl-4,6-di[ethanoyl]dibenzofuran) is a member of the dibenzofuran family and is extracted from *Ramalina terebrata* (Antarctic lichen). We investigated if DB3 exerted an antitumor effect on B16F10 melanoma cells. The results revealed that DB3 exerted time- and dose-dependent reduction of cell viability by inducing apoptosis and significantly suppressing cell proliferation through cell cycle arrest in the G0/G1 phase in B16F10 melanoma cells. Additionally, DB3 impeded the migration and invasiveness of B16F10 cells. Subsequently, we observed that DB3 decreased the expression levels of Cdk4/Cyclin D1 and the phosphorylation of p38, JNK, ERK, and AKT. Furthermore, DB3 decreased melanoma tumor growth in a mouse tumor syngraft model. Based on these findings, we propose that DB3 possesses potential for use as an anti-cancer agent for melanoma treatment.

Keywords Anti-cancer, DB3, Proliferation, Migration, Invasion

Introduction

Melanoma originates from genetic mutations in melanocytes and can manifest in the skin, eye, and inner ear, and it is one of the most aggressive and lethal types of cutaneous cancer [1]. Although melanoma constitutes a small proportion of total skin cancer cases, it is responsible for 80% of related mortalities [2]. The incidence of melanoma is rising more rapidly than that of other cancers, with projections indicating a potential doubling in the number of patients within the next 10–20 years [2]. Melanoma is indistinguishable from dysplastic nevi in terms of its morphological characteristics, thus making it difficult to diagnose in its early stages [3].

Cancer cells, including melanoma cells, are characterized by their ability to proliferate limitlessly and are marked by uncontrolled growth and reduced ability to undergo apoptosis [4, 5]. The diminished apoptosis capability enables cancer cells to survive for longer periods of

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time and continue proliferating as tumors progress [4, 5]. Furthermore, cancer cells possess the capacity for migration and invasion, both of which are critical factors driving metastasis [6, 7]. Consequently, anti-cancer drugs have been developed to suppress proliferation, impede migration and invasion, and induce apoptosis in cancer cells [8]. It is established that a variety of key intracellular signaling proteins regulate cancer processes such as proliferation, apoptosis, migration, and invasion [9]. Most melanoma cases exhibit an aberrant Cyclin D1-CDK4-Rb pathway [10]. Also, it has been reported that the mutated and amplified CDK4 in melanoma caused the dysregulation of cell proliferation [11]. Cyclin D1 and its binding partner CDK4 (Cyclin-dependent kinase4) are crucial components of the cell cycle that facilitate the transition from the G1 phase to the S phase [12]. Moreover, The MAPK and AKT pathways have been known to have significant relevance to the development of melanoma.[13]. MAPK proteins, including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 (p38 mitogen-activated protein kinase), have been demonstrated to contribute to the control of cell proliferation and apoptosis [14, 15]. Most melanoma has BRAF (serine/threonine protein kinase) mutation, which is significantly associated with MAPK signaling pathway [13, 16]. Especially, BRAF mutation in melanoma has been known to activate ERK pathway, causing dysregulated proliferation [16]. The AKT pathway is also known to play a significant role in cellular proliferation [17].

Although some chemotherapies have been developed for melanoma, they often cause adverse side effects [18]. Cisplatin and temozolomide are well-established chemotherapeutic treatments for melanoma [18]. Both drugs reduce cancer cell viability and induce apoptosis by disrupting DNA, RNA, and protein synthesis [19, 20]. Cisplatin therapy induces nausea and vomiting, reduces blood cell and platelet production in the bone marrow, weakens the infection response, and can damage kidneys and neurons and lead to hearing loss [19]. Additionally, Temozolomide's side effects encompass thrombocytopenia, lymphopenia, and neutropenia [21]. To address these issues, numerous research groups are actively working to discover compounds with few or no adverse effects. Therefore, the identification of novel compounds that can reduce these adverse effects is imperative.

Phytochemicals have been identified as safe compounds with minimal toxicity, thus making them promising candidates as complementary therapies for melanoma treatment [22]. Consequently, there has been an increased focus on studying phytochemicals as potential anti-cancer agents capable of reducing the side effects associated with existing chemotherapy [22]. Phytochemicals such as fisetin and genistein have been reported as potential anti-cancer agents [22]. Fisetin is present abundantly in various fruits and vegetables and possesses the potential to inhibit tumor growth by inducing anti-proliferative effects and promoting apoptosis in cancer cells [23]. Genistein is extracted from soybeans and exhibits antitumor properties [24].

DB3, a member of the dibenzofuran family, is a novel compound derived from the Antarctic lichen *Ramalina terebrata* [25]. This lichen produces numerous secondary metabolites with diverse properties [26]. Consequently, it has been used extensively in traditional medicines in the Antarctic regions [26]. Additionally, the dibenzofuran family has not been previously reported for its anti-cancer effects. In this study, we investigated the anti-cancer effects and molecular mechanisms of DB3 in vitro and in vivo.

Materials and methods

Reagents

DB3 (1,3,7,9-tetrahydroxy-2,8-dimethyl-4,6-di[ethanoyl] dibenzofuran) was obtained from the Korean Polar Research Institute. Dulbecco's Modified Eagle medium (DMEM) was purchased from HyClone (Marlborough, MA, USA). Penicillin–streptomycin-glutamine (PSQ) was purchased from Gibco (Waltham, MA, USA). Reagents including 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), crystal violet, DMSO, trypan blue, and paraformaldehyde were obtained from Sigma-Aldrich (St. Louis, MO, USA). DPBS and trypsin-ethylenediaminetetraacetic acid (EDTA) were purchased from WELGENE, Inc. (Gyeongsan, Korea). Carboxyfluorescein diacetate succinimidyl ester (CFSE) and propidium iodide (PI) were obtained from Invitrogen (Carlsbad, CA, USA). Ethanol and Agar were obtained from Emsure (Billerica, MA) and Affymetrix (Santa Clara, CA), respectively. FBS (Fetal bovine serum) were obtained from Corning (NY, USA). FITC-labeled Annexin V was purchased from BD Pharmingen (San Diego, CA, USA). Primary antibodies (p-AKT, p-JNK, p-ERK, p-p38, and β-actin) were obtained from Cell Signaling Technology (Danvers, MA, USA), while Cdk4 and Cyclin D1 were acquired from Santa Cruz Biotechnology (Dallas, TX, USA). Secondary antibodies (goat anti-mouse and goat anti-rabbit IgG) were purchased from SeraCare (Gaithersburg, MD, USA).

Cell culture

B16F10 melanoma cells were obtained from Dr. Kwang Dong Kim at Gyeongsang National University in Jinju, Korea, and cultured in DMEM supplemented with 10% FBS and 1% PSQ.

Cell viability assay

Approximately 5×10^4 cells were seeded into each well of a 48-well plate. After 24 h, various concentrations of DB3 were administered. Subsequently, at 24 and 48 h post-treatment, the media were removed, and 1X MTT solution was added. This was followed by incubation for 2 h at 37 °C in a CO_2 environment. After incubation, DMSO was added to each well to dissolve formazan crystals. Absorbance was measured at 570 nm using a Varioskan LUX Multimode Microplate Reader (Thermo Fisher Scientific).

Trypan blue exclusion assay

Approximately 2×10^4 cells were seeded into each well of a 12-well plate. After 24 h, various concentrations of the DB3 were added to each well. At 24 and 48 h post-treatment, the cells were detached using trypsin–EDTA and stained with Trypan Blue. The cell counts were determined using a hemocytometer.

Colony formation assay

Approximately 5×10^2 cells were seeded in each well of 6-well plates. After 24 h, 25 μM DB3 was added to each well. After six days, the colonies were fixed with 4% paraformaldehyde for 10 min and subsequently stained with 0.05% crystal violet. Following staining, each well was washed with PBS, and the plate was inverted to allow drying for approximately 1–2 days. Once the plate was completely dry, crystal violet was dissolved in 10% acetic acid, and the absorbance was measured at 570 nm using a Varioskan LUX Multimode Microplate Reader (Thermo Fisher Scientific).

Apoptosis assay

Approximately 4×10^4 cells were seeded into each well of a 6-well plate. After 24 h, 25 μM DB3 was added to each well. After 24 and 48 h of treatment, the cells were harvested and stained with both PI (Propidium Iodide) and FITC-labeled annexin V. The analysis was conducted using Flow cytometry (BD FACSVerse™, BD Biosciences).

CFSE Cell proliferation assay

Approximately 4×10^4 cells were seeded into each well of a 6-well plate. After 24 h, the cells were stained with CFSE, and then 25 μM DB3 was added to each well. The cells were fixed with 4% paraformaldehyde and harvested 0, 12, 24, and 48 h after treatment. The analysis was performed using Flow cytometry (BD FACSVerse™, BD Biosciences).

Cell cycle analysis assay

Approximately 4×10^4 cells were placed into each well of 6-well plates. After 24 h, 25 μM DB3 was added to each well. At 24 and 48 h after treatment, the cells were harvested along with the supernatant. The cells were fixed with 70% ethanol overnight at 4 °C, and then were stained with PI. The cell cycle analysis was conducted using Flow cytometry (BD FACSVerse™, BD Biosciences).

Cell migration assay

For the wound healing assay, approximately 5×10^5 cells were seeded into each well 6-well plates. After 24 h, the wells were scratched using blue tips, and then 25 μM DB3 was added to each well. At 0, 12, and 24 h after scratching, well images were captured using a microscope, and the gap was analyzed using Image J software.

Cell invasion assay

For the soft agar assay, a 0.3% agar layer in the upper portion of the plate was prepared, and each well was supplemented with 5×10^3 cells and 25 μM DB3. The lower agar layer contained 0.6% agar. The plate was then incubated for 3 weeks in a 37 °C incubator. Subsequently, the colony sizes were analyzed using a microscope.

Western blot analysis

Approximately 2×10^5 cells were seeded into 60 mm culture dishes. After 24 h, 25 μM DB3 was added to each dish. At 24 and 48 h post-treatment, the cells were harvested and lysed using RIPA buffer (1% Triton X-100, 1% sodium deoxycholate, 1 mM EDTA Ph 8, 10 mM Tris Ph 7.4, 150 mM NaCl) supplemented with phosphatase inhibitors (1.5 mM $NaVO_4$ and 30 mM NaF) and protease inhibitors (1 mg/mL aprotinin, pepstatin A, leupeptin, and 1 mM PMSF). Proteins were separated by SDS-PAGE and transferred onto an NC (nitrocellulose) membrane. Ponceau's solution was used to ensure proper protein transfer. Subsequently, the NC membrane was blocked with 5% skim milk in Tris-buffered saline (TBS), 0.1% Tween-20, and 0.01% NaN_3. The primary antibodies were dissolved in the same blocking solution, and the membranes were incubated overnight for attachment. Subsequently, the membranes were washed with Tris-buffered saline (TBS) containing 0.1% Tween-20. The membranes were incubated with secondary antibodies prepared in 5% skim milk without 0.01% $NaN_{3,}$ and the membranes were incubated to attach the secondary antibodies. The membranes were washed with Tris-buffered saline (TBS) containing 0.1% Tween-20. Finally, an ECL solution

was applied to the membranes, and the proteins were detected using Chemidoc (iBright™ CL1500, Invitrogen).

Subcutaneous murine tumor model of B16F10 cells

Male C57BL/6 mice (8 weeks of age) were obtained from Koatech (Jinju, Korea) and used for the experiments. To investigate tumorigenesis in vivo, B16F10 cells were initially cultured in 100 mm culture dishes. After 24 h, 25 μM DB3 was added to the plates. The cells were harvested using trypsin–EDTA and suspended in PBS. The mice were divided into two groups for the melanoma model. Mice from group I were injected with 5×10^5 B16F10 cells subcutaneously into the right flank. Group II mice were injected with 5×10^5 B16F10 cells treated with DB3 subcutaneously into the right flank (n = 3 per group). Three weeks after injection, the mice were sacrificed, tumors were extracted, images were acquired, and tumors were quantitatively analyzed using the ImageJ program. The Institutional Animal Care and Use Committee at Gyeongsang National University approved this experimental protocol used in this study (GNU-221216-M0181-01).

Statistical analysis

Data are presented as three independent replicates. The results are presented as mean ± standard deviation (SD). Differences between groups were analyzed using the Student's t-test. Statistical significance is indicated by $^*p < 0.05$.

Results

DB3 reduced the viability of B16F10 melanoma cells

To investigate the effect of DB3 on cell viability in B16F10 cells, an MTT assay was performed at various concentrations of DB3 (0, 15, 25, and 50 μM) for 24 and 48 h. The cell viability decreased in a time- and dose-dependent manner (Fig. 1B). Also, the trypan blue exclusion assay was conducted at different concentrations of DB3 (0, 15, 25, and 50 μM) for 24 and 48 h. The cell numbers decreased in a time- and dose-dependent manner (Fig. 1C). Additionally, we performed a colony-forming assay with DB3 treatment for 6 days in B16F10 melanoma cells. There was a decrease in colony-forming ability compared to that of the control group (Fig. 1D and E). These results demonstrated that DB3 decreased cell viability.

DB3 slightly promoted apoptosis of B16F10 melanoma cells

Previous studies have suggested that anti-cancer drugs possessing cytotoxic effects can induce programmed cell death [27, 28]. We investigated if DB3 affected apoptosis in B16F10 melanoma cells. The results of the flow cytometry analysis revealed an increase in PI-negative and Annexin V-positive areas in the DB3-treated group, indicating an apoptotic cell population (Fig. 2A). After DB3 treatment for 24 and 48 h, the proportion of PI-negative and Annexin V-positive cells undergoing apoptosis was slightly increased compared to levels in non-treated cells.

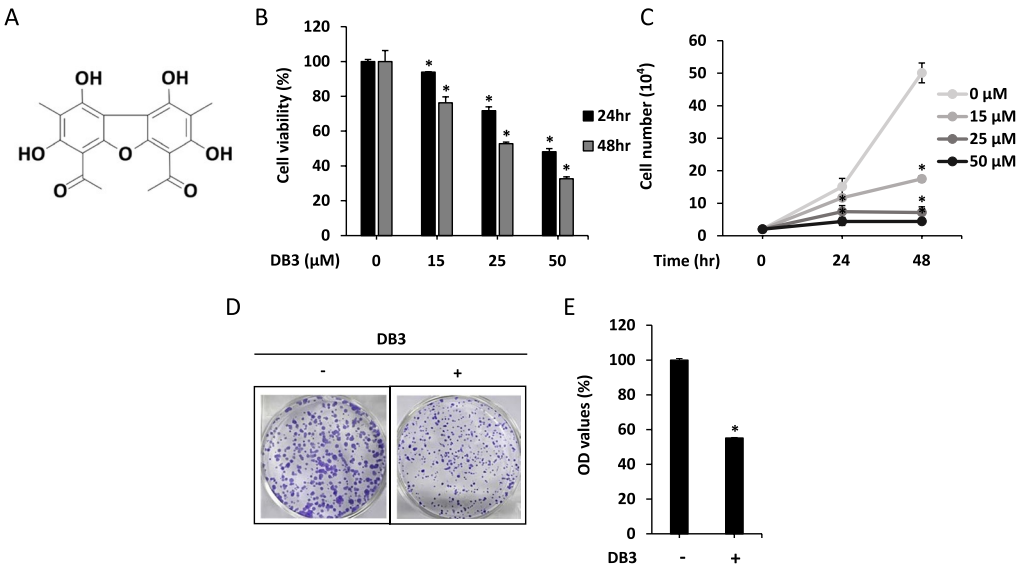

Fig. 1 DB3 reduced the viability of B16F10 melanoma cells. **A** Structure of DB3. **B** After 24 and 48 h of treatment with DB3 at 0, 15, 25, and 50 μM, cell viability was analyzed by MTT. **C** Cell numbers were analyzed by Trypan blue exclusion assay. **D** A colony-forming assay was performed after 6 days of 25 μM DB3 treatment to determine cell viability and proliferation ability. **E** The graph presents the percentage of OD values. $^*p < 0.05$ when compared to control. The data are presented as the mean ± SD. Three independent experiments were performed, and representative data are presented

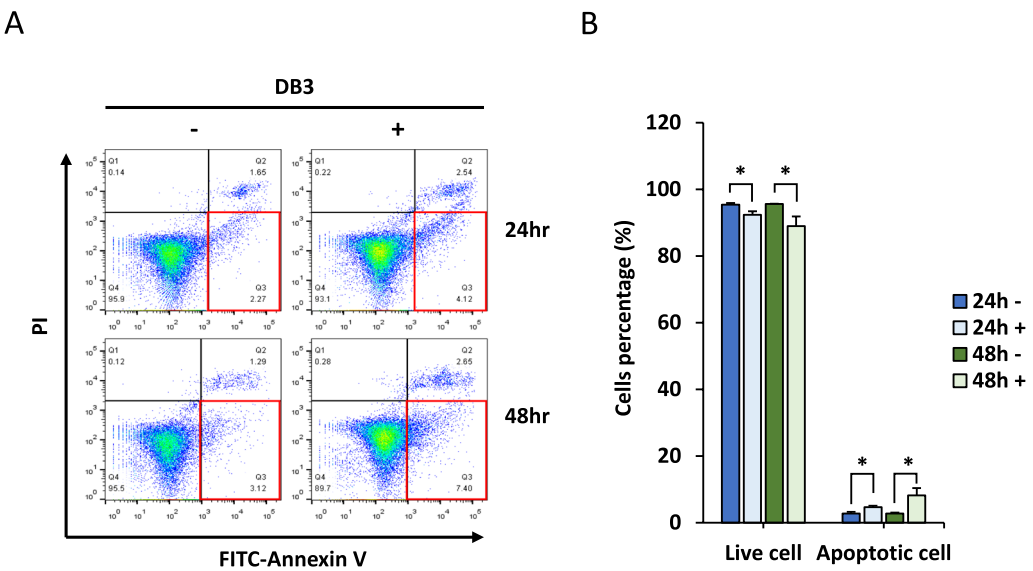

Fig. 2 DB3 promoted apoptosis of B16F10 melanoma cells. After 24 and 48 h of 25 μM DB3 treatment, cells were analyzed by flow cytometry. **A** The PI and FITC-Annexin V stained cells were analyzed by flow cytometry. **B** The graph presents the percentage of populations of live cells and apoptotic cells. *$p < 0.05$ when compared to the control group. The data are presented as the means ± SD. Three independent experiments were performed. Among them, representative data are presented

(Fig. 2B). These findings suggested that DB3 induces apoptosis in B16F10 melanoma cells.

DB3 inhibited the proliferation of B16F10 melanoma cells

Cell proliferation refers to an increase in cell number via cell division [29]. The inhibition of cell proliferation is attributed to a decrease in cell viability [30]. The effects of DB3 on the proliferation of B16F10 melanoma cells were also investigated. Following the staining of the cells with CFSE and subsequent analysis using flow cytometry, we observed that the CFSE intensity of DB3-treated cells was higher than that of the untreated cells (Fig. 3A). These findings implied that DB3 treatment inhibited the proliferation of B16F10 cells.

DB3 induced G0/G1 cell cycle arrest in B16F10 melanoma cells

Cell cycle arrest is related to proliferation, as regulation of the cell cycle directly affects proliferation ability and can lead to its deregulation, thus potentially contributing to cancer [31–33]. Therefore, we investigated if DB3 affects the cell cycle in B16F10 melanoma cells by inducing cell cycle arrest. Our results revealed that B16F10 melanoma cells treated with DB3 exhibited an increase in the G0/G1 phase compared to levels in non-treated cells (Fig. 3B and C). Additionally, we observed a decrease in the G2/M phase in DB3-treated cells compared to that in untreated cells (Fig. 3D). These results indicate that DB3 inhibits the proliferation of B16F10 melanoma cells by inducing cell cycle arrest in the G0/G1 phase.

DB3 reduced the migration of B16F10 melanoma cells

The migratory ability of cancer cells plays an important role in metastasis [6]. In particular, melanomas can migrate to form malignant melanomas [7]. We investigated if DB3 affected the migration of B16F10 melanoma cells. In the wound healing assay, we created a scratch on the cell layer and monitored cell movement in two dimensions while preserving the cell-to-cell junctions. The wound gap in cells treated with DB3 did not close even after 12 and 24 h, thus indicating an inhibitory effect of DB3 on cell migration (Fig. 4A and B). These results indicate that DB3 reduces cell migration ability.

DB3 inhibited the invasion of B16F10 melanoma cells

In addition to migration, invasion is significantly associated with melanoma metastasis and is a hallmark of malignant cancers [7, 34]. We conducted a soft agar assay that is a widely recognized in vitro technique for assessing invasive potential to demonstrate the malignant transformation of these cells [35]. Cells possessing anchorage-independent capacity can grow continuously on agar and form colonies [35]. The colony size of cells treated with DB3 decreased after three weeks, thus indicating an inhibitory effect of DB3 on cell invasion (Fig. 5A and B). These results indicate that DB3 inhibits cell invasion.

DB3 inhibited the phosphorylation of MAPK and AKT in B16F10 melanoma cells

The MAPK and AKT signaling pathways are key signaling pathways involved in proliferation [36]. Western

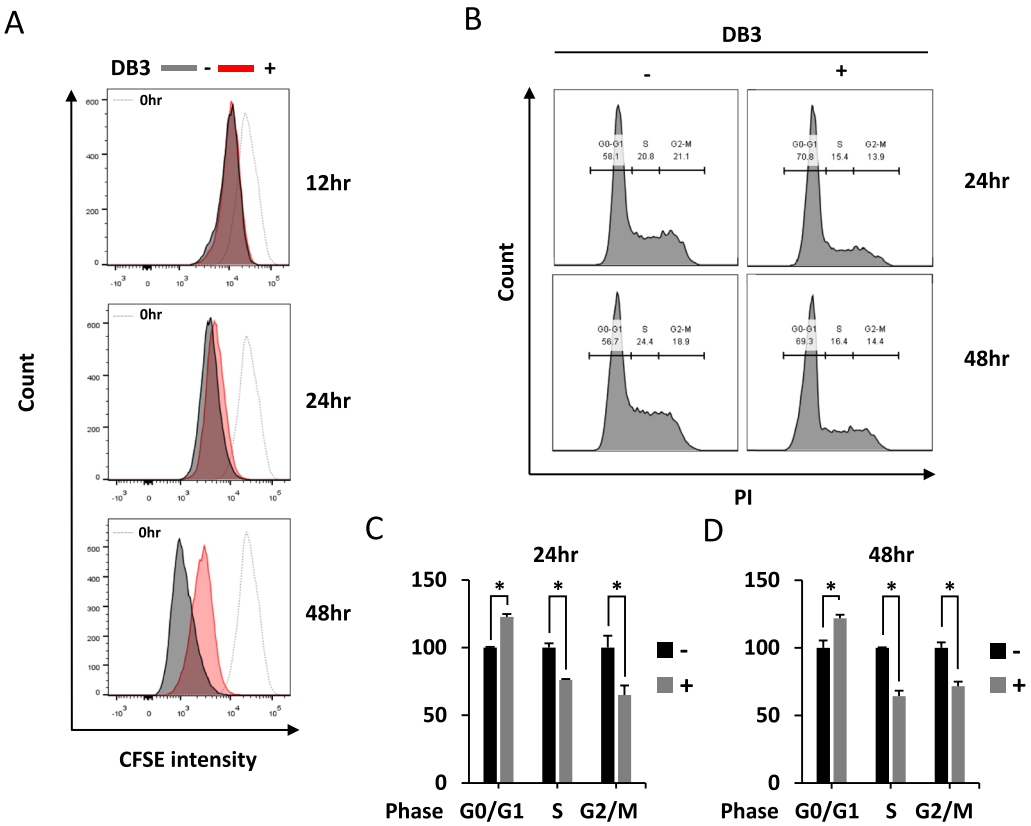

Fig. 3 DB3 inhibited the proliferation of B16F10 melanoma cells. The CFSE cell proliferation assay and cell cycle assay were performed using Flow cytometry **A** The CFSE cell proliferation assay was analyzed after 12, 24, and 48 h of 25 μM DB3 treatment. **B** The histogram presents the cell cycle analysis. **C**, **D** The graph represents a percentage of each cell cycle phase. *$p < 0.05$ when compared to the control group. The data are presented as the means ± SD. Three independent experiments were performed. Among them, representative data are presented

blot analysis confirmed that the phosphorylation levels of ERK, p38, JNK, and AKT were decreased following DB3 treatment (Fig. 6A). These results indicate that the MAPK and AKT pathways are associated with the proliferation of B16F10 melanoma cells.

DB3 downregulated the expression levels of cell cycle-related proteins in B16F10 melanoma cells

Previous studies have reported that G0/G1 phase arrest of the cell cycle is associated with a decrease in Cdk4 and Cyclin D1 proteins [31]. The expression levels of Cdk4 and Cyclin D were decreased by DB3 treatment (Fig. 6B). These results indicate that DB3 could induce cell G0/G1 phase arrest by regulating the expression levels of Cdk4 and Cyclin D1.

DB3 reduced tumor growth in the in vivo system.

We investigated the effects of DB3 on tumor growth in a murine tumor syngraft model. DB3-treated or untreated B16F10 cells were injected into the right flank of the mice and allowed to grow to approximately 2000 mm^3 (Fig. 7A and D). We confirmed that DB3 reduced tumor size in vivo (Fig. 7B and C), thus indicating that DB3 can inhibit tumor growth in vivo.

Discussion

Although melanoma constitutes a small proportion of skin cancer cases, it is responsible for 80% of related mortalities [2]. The incidence of melanoma may result in a doubling of the number of patients within the next 10–20 years [2]. Although various drugs have been proposed for the treatment of melanoma, they are often accompanied by numerous adverse side effects [18–21]. For example, chemotherapeutic agents such as Temozolomide and Cisplatin that have traditionally been employed for the management of melanoma can cause severe side effects [19–21]. Therefore, there is an urgent need to identify novel compounds that can reduce these adverse effects.

Phytochemicals cause few side effects, thus making them ideal agents for melanoma treatment [22]. DB3 was extracted from the Antarctic lichen *Ramalina terebrata* which is used for traditional medicine in Antarctic regions [25, 26]. DB3, a member of the dibenzofuran

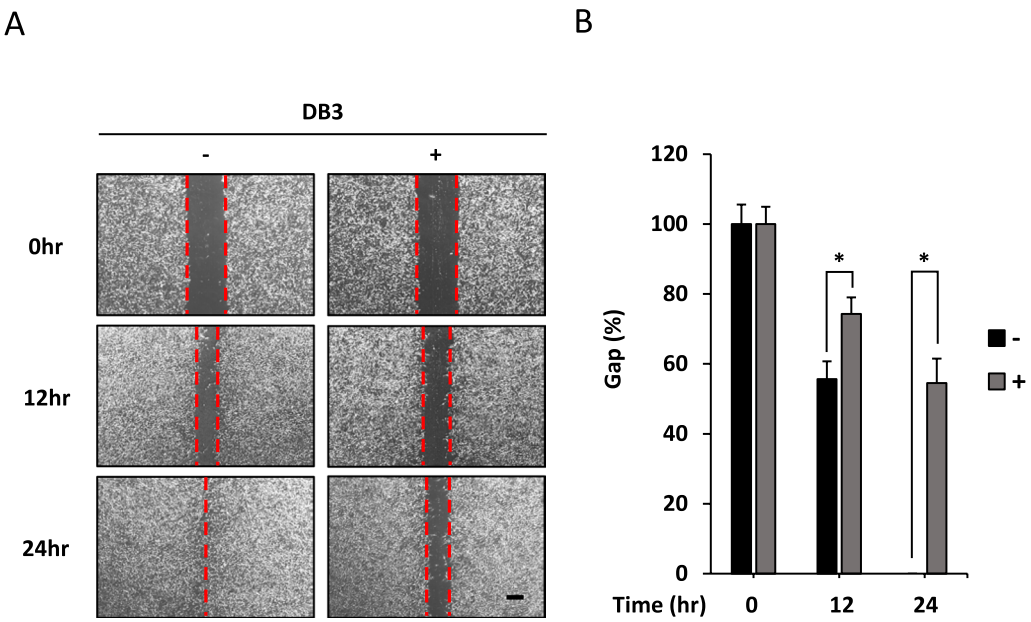

Fig. 4 DB3 reduced the migration of B16F10 melanoma cells. **A** A wound-healing assay was performed after 12 and 24 h of 25 μM DB3 treatment, and images were acquired after scratching wells filled with B16F10 melanoma cells. Scale bar: 200 μm **B** Quantitative analysis of panel A. *$p < 0.05$ when compared to the control group. The data are presented as the means ± SD. Three independent experiments were performed. Among them, representative data are presented

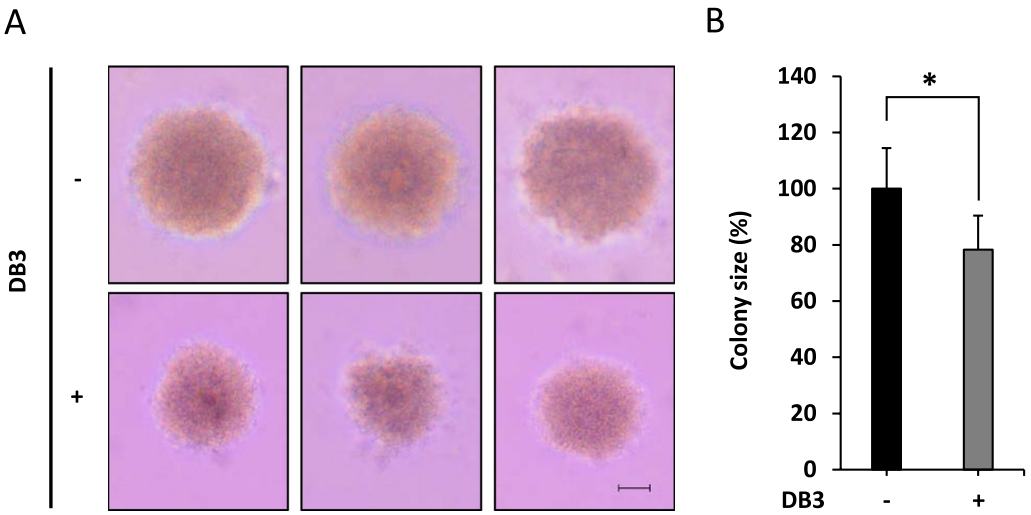

Fig. 5 DB3 inhibited the invasion of B16F10 melanoma cells. **A** The soft agar assay was analyzed after 21 days of 25 μM DB3 treatment, and images were acquired using a microscope. Scale bar: 20 μm **B** Colony size is presented by the graph. *$p < 0.05$ when compared to the control group. The data are presented as the means ± SD. Three independent experiments were performed. Among them, representative data are presented

family, has not been previously reported for its biological activity anti-cancer effects, and mechanisms. In this study, we investigated if DB3 could exert anti-cancer effects on B16F10 melanoma cells. In the investigation of the mechanisms by which DB3 decreased cell viability, we observed that DB3 induced apoptosis and inhibited proliferation by increasing G0/G1 cell cycle arrest. These results suggested that DB3 reduced cell viability by inhibiting proliferation and inducing apoptosis. We also revealed that DB3 can inhibit cell migration and invasion, both of which are major functions of metastasis in cancer cells. Moreover, we determined that DB3 reduced tumor growth in vivo.

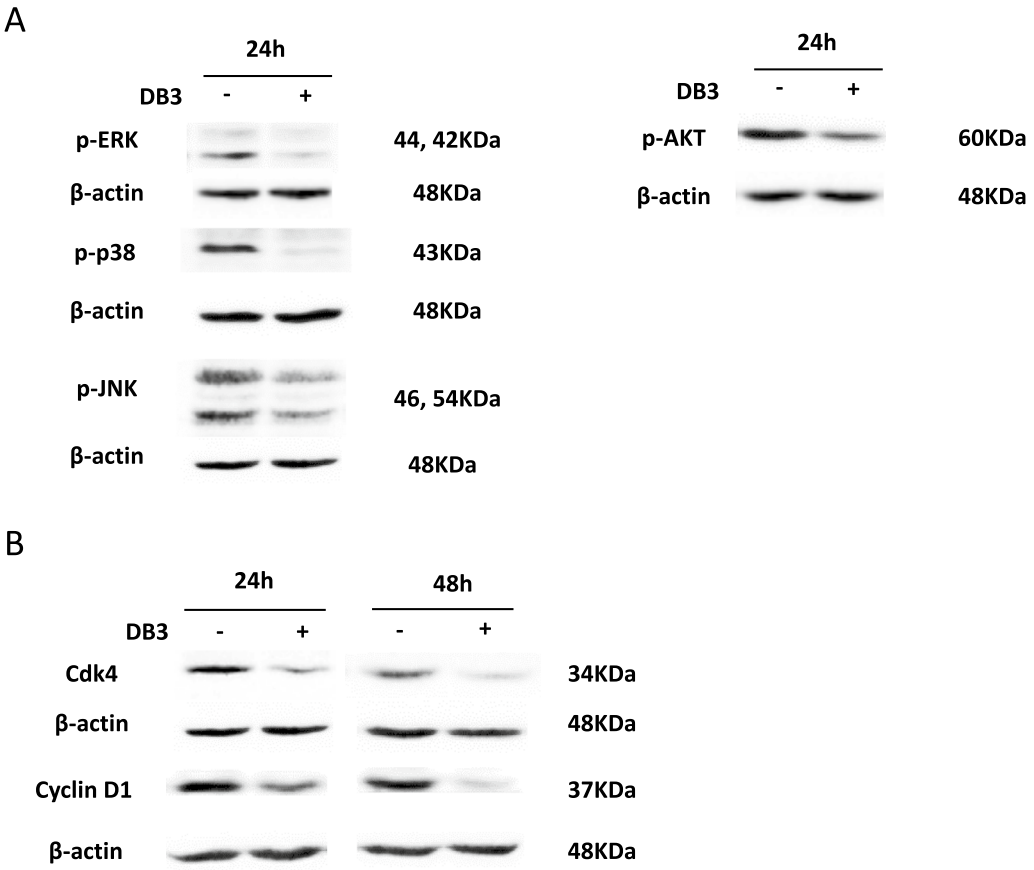

Fig.6 DB3 reduced the phosphorylation levels of the MAPK/AKT signaling pathway members and the protein expression levels associated with the G0/G1 phase. **A** Phosphorylation levels of MAPK and AKT were analyzed. **B** Protein expression levels associated with the G0/G1 phase were analyzed by western blotting

Subsequently, we investigated the effects of DB3 on MAPK and AKT signaling pathways in B16F10 melanoma cells. MAPK and AKT signaling are known to be involved in various biological functions, including cell proliferation, apoptosis, migration, invasion, and cell cycle regulation, and they have also been associated with tumorigenesis [36–38]. The ERK phosphorylation is increased in various tumors and stimulates various downstream molecules to induce cell proliferation [36, 38]. The JNK signaling pathway is implicated in a range of physiological processes, including cellular transformation in cancer cells [39]. Additionally, overexpression has been observed in various cancer cell types [39]. The p38 signaling pathway also plays a significant role in proliferation, apoptosis, cell migration, and tumorigenesis [40]. The AKT signaling pathway also referred to as the protein kinase B (PKB) pathway, plays crucial roles in cell proliferation, apoptosis, and growth [41]. Overexpression of AKT, an oncogenic protein, has been observed in numerous cancer cell lines [41]. Currently, numerous anti-cancer drugs are designed to inhibit the MAPK and AKT pathways [41]. DB3 reduced MAPK and AKT phosphorylation. These results suggest that DB3 exerts anti-cancer effects by downregulating MAPK and AKT phosphorylation levels.

Cdk4 and Cyclin D1 have been known to regulate the transition from G1 to S phase [12]. Cyclin D1 is overexpressed in tumor cells [12]. Growth factors induce the binding of Cdk4 to Cyclin D1, thus leading to the phosphorylation of the retinoblastoma protein (Rb), a tumor suppressor [42]. The phosphorylation of Rb results in the detachment of E2F that interacts with Rb. This process activates gene transcription, thereby driving the transition from the G1 to the S phase [42]. Therefore, we suggest that G0/G1 cell cycle arrest in response to DB3 is mediated by the downregulation of Cdk4 and Cyclin D1.

In summary, our findings indicate that DB3 inhibits the phosphorylation of MAPK and AKT and also the expression of Cdk4 and Cyclin D1, thus resulting in the induction of apoptosis, inhibition of proliferation, and cell cycle arrest at the G0/G1 phase. These outcomes suggest that DB3 possesses potential as a

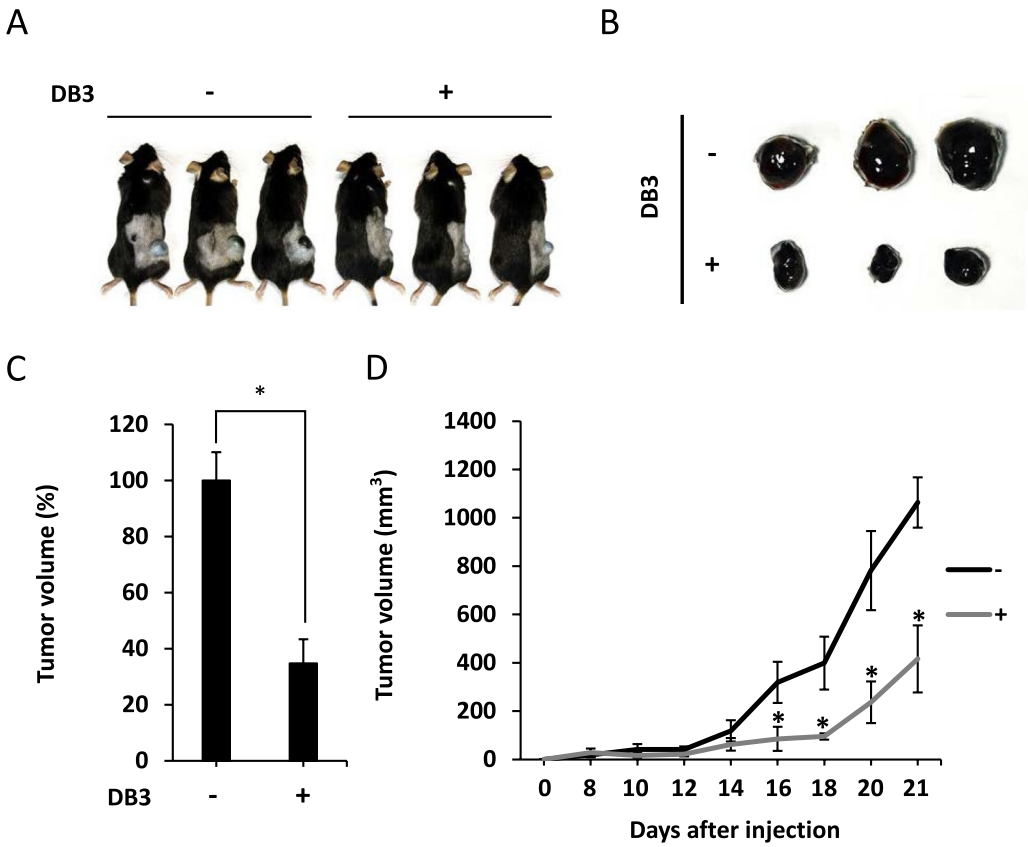

Fig.7 DB3 reduces tumor size in vivo. **A** Images of tumor-bearing mice after injecting DB3-treated or non-treated B16F10 cells. **B** Images of reduced tumor size following DB3 treatment. **C** Quantitative analysis of panel B. **D** The graph represents the tumor size development after injecting the B16F10 melanoma cells. *$p < 0.05$ when compared to the control group. The data are presented as the means ± SD. Three independent experiments were performed. Among them, representative data are presented

new, low-side-effect anti-cancer drug for the treatment of melanoma by regulating cell proliferation and apoptosis.

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Author contributions
SC, HK, S-AS, MK, MK, and SL performed the experiments. SC wrote the manuscript under the guidance of SYM, UJY, and CSL SYM, JHL, HHP, UJY. provided intellectual contributions to this study. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials
The datasets that support the finding of this study are available from the corresponding author on reasonable request.

Declarations

Competing interests
The authors declare no competing interest.

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