Deguelin inhibits the migration and invasion of lung cancer A549 and H460 cells via regulating actin cytoskeleton rearrangement

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Abstract: Deguelin, the main components from Mundulea sericea, was reported to suppress the growth of various cancer cells. However, the effect of Deguelin on tumor cell invasion and metastasis and its mechanism still unclear so far. In this study, we investigated the effects of Deguelin on the cell invasion in human lung cancer A549 and H460 cells. Our results demonstrate that Deguelin can significantly inhibited cell proliferation, cell migration and cell invasion. Moreover, Deguelin could also affected reorganization of the actin cytoskeleton and decreased filopodia and lamellipodia formation. Furthermore, deguelin-treated tumors showed decreased the tumor metastasis related genes such as CD44, MMP2 and MMP9 at protein and mRNA levels and the content of CEA, SCC, NSE, CY-FAR21-1. In addition, Deguelin down-regulated protein expression of Rac1 and Rock1, which are impotent in actin cytoskeleton rearrangements and cell motility. Together, our results suggest that Deguelin inhibit tumor growth and metastasis of lung cancer cells and might be a candidate compound for curing lung cancer.

Keywords: Lung cancer, invasion, Deguelin-actin, cytoskeleton, rearrangement

Introduction

Lung cancer is the leading cause of cancer related death all over the world, and non-small cell lung cancer (NSCLC) is the most common type of lung cancer [1]. Many patients diagnosis of lung cancer are at advanced stages, and the prognosis of these patients remains very poor. Tumor metastasis is the most cause of death for lung cancer patients, metastasis is diagnosed in approximately 30% patients with lung cancer, among which, relapse and metastasis may occur in 50%-60% of the patients with operable lung cancer [2]. Despite the improvements made in surgical and chemotherapeutic modalities to combating lung cancer, the 5-year survival rate still remains relative low [3]. Thus, it is urgent to identify a new anticancer drug targeting to prolong survival and improve quality of life for lung cancer patients.

Deguelin, a rotenoid extracted from Mundulea sericea, was found to have anti-tumor effect activity in various human cancer cell types, including breast cancer, prostate cancer, colorectal cancer and lung cancer [4-7]. Deguelin was reported to arrest the cell cycle at the S phase and induce cell apoptosis in the MDA-MB-231 cells [4]. Deguelin induced the generation of ROS by inhibiting the PI3K/Akt pathway [8]. Deguelin suppressed pancreatic tumor growth and metastasis by inducing apoptosis and inhibiting epithelial to mesenchymal transition [9]. Degulin had been reported to affect different types of human cancer cells growth, but its anti-metastatic effects remains unclear. In the present study, we aimed to evaluate the effects of deguelin on cell migration and invasion in human lung cancer A549 cells and its molecular mechanisms.

Materials and methods

Cell culture and drug treatment

Human lung cancer A549 and H460 cell line was obtained from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China), cultured in DMEM (Hyclone,
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Logan, USA) containing 100 U/ml penicillin and 10% fetal bovine serum (FBS) at 37°C in 5% CO₂. When the growth of cell monolayer reached 70%-80% confluence, 0.25% trypsin was used for digestion and passaging.

**Cell viability assay**

The effect of Deguelin on cell viability was determined using the Cell Counting Kit-8 (CCK-8, Dojindo Laboratories, Kumamoto, Japan). After 48 h incubation, A549 and H460 cells were treated with 0, 2 μM, 5 μM, 10 μM, 15 μM, 20 μM, 25 μM, 30 μM of Deguelin for 48 h, and then the cells were added with 10 μL CCK-8 solution for additional 2 h incubation at 37°C, and plates were read with a microplate reader at 450 nm. The percentage of cell viability was calculated against control.

**Cell invasion and migration assays**

The transwell chamber invasion assay employed for cell migration. Briefly, Transwell membrane coated with 2.5 mg/mL fresh Matrigel, followed by incubation at 37°C for 1 h. A549 and H460 cells were starved for 24 h, and then cells were trypsinized, centrifuged, and resuspended at 10⁷ cells/mL in FBS-free DMEM. 5x10⁵ tumor cells in 200 μl of serum-free mediums was added to the upper chamber, while the lower wells of the transwells contained 600 μl DMEM medium with 10% FBS. After incubated for 24 h, the chamber was removed, wiped off the cells on Matrigel and surface of the supper chamber carefully with a cotton swab. Fixed with 4% paraformaldehyde at room temperature for 10 min and stained with 0.5% crystal violet. The cells were counted and photographed with a light microscope at 200×. The cell invasion assay was performed as described except Matrigel was not coated. Randomly select the central field and 6 peripheral fields to count the cells. Independent experiments were repeated for 3 times.

**Real-time quantitative reverse transcription-PCR**: After treated with 10 μM and 20 μM Deguelin for 48 h. Total RNA was isolated with TRIzol reagent (Invitrogen) according to the manufacturer's instructions. 2 μg total RNA was added to synthesize the cDNA according to the protocol of M-MLV reverse transcription kit. The reaction conditions were: 2 min at 50°C, 10 min at 95°C, and 40 cycles of 15 s at 95°C; 1 min at 60°C using the SYBR Green PCR Master Mix (Applied Biosystems) and ABI 7900 sequence detection system. Analyze the melting curve of amplified PCR product; in the meantime, randomly carry out agarose gel electrophoresis (2% volume fraction) to determine whether the product was the amplified target fragment. The relative expression level of mRNAs was determined by 2⁻¹ΔΔCT method with GAPDH as the internal reference. The PCR primer sets were synthesized and purified by Beijing Genomics Institute. CD44 primers: forward CGTGAATACCATCGCAAAAG and reverse CGGACACCAGGACAGTGT; MMP2 primers: forward AAGAAGTATGCAGACCCGCC and reverse TTGCTGGAGACAAATTCTGG; MMP9 primers: forward GACTGAGGATGTCAAGGG and reverse ACGACGTCTCCGCCATACC GA.

**Western blot**: A549 and H460 cells were grown in six well plate and treated with Deguelin (10 and 20 μM) for 48 h. The cells were washed with ice-cold PBS for three times and lysed with RIPA lysis buffer for 30 minutes at 4°C. The protein content was measured using the BCA method. The proteins were separated on 10% SDS-PAGE and transferred onto PVDF membrane. The blots were probed with antibodies specific for mouse anti-CD44 (1:400), mouse anti-MMP2 (1:400) and mouse anti-MMP9 (1:400, from Santa Cruz Biotechnology), and mouse anti-Rac1 (1:1000, BD Biosciences) and rabbit anti-ROCK1 (1:1000, EMD Millipore) at 4°C overnight and subsequently incubated with specific HRP-streptavidin-conjugated secondary antibody for 1 h. Signals were visualized using ECL Substrates (Amersham, Buckinghamshire, UK). β-actin was used as an endogenous protein for normalization.

**Immunofluorescence staining**: A549 cells treated with Deguelin (10 and 20 μM) for 48 h, and then washed twice with pre-cooled PBS (pH 7.4). The cells were fixed with 4% paraformaldehyde at room temperature for 30 min and then gently washed with PBS for 5 min ×3 times, followed by treatment with 0.5% TritonX-100 solution at room temperature for 30 min, washes with PBS for 3 min ×2 times, blocking with 3% skim milk powder (prepared with PBS) at 4°C for 1 h, and again washes with PBS for 5 min ×3 times in order of sequence. The cells were incubated with 5 μg/ml rhodamine-phalloidin (labeling F-actin) at room tem-
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![Graphs showing cell viability](image)

**Figure 1.** Deguelin inhibits the proliferation of lung cancer cells. A549 and H460 cells were treated with various concentrations of Deguelin for 48 h. Cell viability was detected using a CCK-8 kit.

Temperature away from light for 30 min, followed by 3 rapid washes with PBS. The nuclei were stained with 200 nmol/L DAPI-PBS at room temperature for 5 min, followed by washes with PBS for 5 min ×3 times. Finally, anti-fade mounting medium was added drop-wise to complete the mounting. The changes in cell morphology were observed under a fluorescence microscope.

**Scanning electron microscopic observation**

A549 and H460 cells were seeded onto autoclaved clean coverglass in six well plate and treated with Deguelin (10 and 20 μM) for 24 h. Then the cell-growing coverglass was washed 3 times with PBS and fixed in 2.5% cold glutaraldehyde, followed by standing at 4°C, 3 washes with PBS and then fixation with 1% osmic acid at 4°C for 1 h. After discarding the fixative solution, the coverglass was washed 3 times with PBS, dehydrated with gradient ethanol, substituted with isomyl acetate, dried at CO₂ critical point, sprayed with gold, observed under a SEM, and photographed in order of sequence.

**ELISA assay**

Cells were seeded in 6-well plates; transfection intervention was carried out after the attached cells entered in logarithmic growth phase. In 48 h after the intervention, the culture media was refreshed for another 24 h, and then the content of CEA, SCC, NSE, CYFAR21-1 was measured by ELISA assay in supernatant.

**Statistical analysis**

All data from three independent experiments were expressed as mean ± SD and processed using the IBM SPSS Statistics software (version 21). *P* values of <0.05 were considered statistically significant.

**Result**

**Deguelin inhibits the proliferation of lung cancer cells**

To assess the anti-proliferation effect of Deguelin on lung cancer cells, we treated lung cancer cell lines A549 and H460 with different concentration Deguelin for 48 h. As showed in Figure 1, Deguelin treatment resulted in cell viability significant inhibition in both A549 and H460 cells.

**Deguelin inhibits cancer cell migration and invasion**

Transwell chamber invasion assay results showed that, when compared with the control group, the migration and invasion of A549 and H460 cells significantly inhibited in a dose-dependent manner in Deguelin treated group. To evaluate the effect of Deguelin on migration and invasion, we selected 10 μM and 20 μM concentrations for below experiments. It was shown that Deguelin suppresses migration and invasion of A549 and H460 cells significantly (Figure 2).
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Deguelin affects reorganization of the actin cytoskeleton

It is widely understood that reorganization of the cytoskeleton, including actin filaments and microtubules, plays a crucial role in cell invasion and migration [10]. We investigated the capacity of Deguelin to regulate actin cytoskeleton in lung cancer cells motility by staining F-actin using fluorescence microscopy. As shown in Figure 3, after treated with Deguelin (10 and 20 μM) for 24 h, F-actin filaments was disorganization, filopodia and lamellipodia were significantly increased in A549 and H460 cells. In order to further investigate the morphological changes of cells by Deguelin, we also detected the morphological by scanning electron microscope (SEM). We found that the number of microvilli significantly decreased after Deguelin treated in A549 and H460 cells (Figure 4). Taken together, Deguelin dire organization of the cytoskeleton by down-regulating filopodia and lamellipodia.

Deguelin regulates cell migration and invasion might through CD44, MMP2 and MMP9

To further study the mechanism of Deguelin-inhibited cell migration and invasion, we examined the changes in related proteins. The results showed that, after treated by Deguelin, significantly decreased the levels of CD44, MMP-2 and MMP-9 in A549 and H460 cells (Figure 5).

Deguelin regulates actin cytoskeleton rearrangements might via Rac1 and ROCK1

Rho-GTPase plays an important role in the actin cytoskeleton rearrangements. To determine whether Deguelin may affect the expression of Rho-GTPase in lung cancer cells, we investigated the protein expression of Rac1 and ROCK1.
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Figure 3. Deguelin affect reorganization of the actin cytoskeleton in A549 and H460 cells. After treated with Deguelin for 24 h, Cells were stained with phalloidin (green) for F-actin, and DAPI fluorescence for DNA (20×).

Figure 4. Deguelin affect the morphologic in A549 and H460 cells. After treated with Deguelin for 24 h, cells were detected by Scanning electron microscopy (5000×). Scale bars: 10 μm.
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As showed in Figure 6, Rac1 and ROCK1 expression decreased after Deguelin treatment in A549 and H460 cells.

Deguelin regulates content of CEA, SCC, NSE, CYFAR21-1 in supernatant of lung cancer cells

CEA, SCC, NSE, CYFAR21-1 were the marker of lung cancer and we found that there was a decreased content of CEA, SCC, NSE, CYFAR21-1 in supernatant of lung cancer cells by Deguelin treatment (Figure 7).

Discussion

Invasion and metastasis of lung cancer are thought to be responsible for the progression and recurrence of lung cancer multiforme. And more than 80% of lung cancer patients died because of tumor invasion and metastasis [11]. Therefore, an agent that could efficiently inhibit the migration and invasion of cancer cells would be a useful candidate to suppress cancer progression and metastasis. Many natural products have been reported to be anti-tumor activity for various cancers [12].

In the current study, Deguelin exerted to suppress tumor growth and metastasis of lung cancer cells in vitro. The underlying mechanisms in this process involved the suppression of CD44, MMP-2 and MMP-9 activity. CD44 is surface antigens associated with tumor metas-
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Figure 6. Deguelin regulates the expression of Rac1 and ROCK1 in A549 and H460 cells. After treated with Deguelin for 24 h, the expression of Rac1 and ROCK1 in A549 and H460 cells were detected by western blot analysis, β-actin used as an internal control.

Figure 7. Deguelin down-regulates content of CEA, SCC, NSE, CYFAR21-1 in supernatant of lung cancer cells.

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Deguelin inhibits lung cancer migration and invasion. CD44 is a receptor for hyaluronic acid and can also interact with other ligands, such as osteopontin, collagens, and matrix metalloproteinases (MMPs) [13]. MMP-2 and MMP-9 are central players in cancer cell migration and invasion [14, 15]. Reduction of MMP-2 and MMP-9 activity may contribute to inhibition of invasiveness of lung cancer cells. Our results suggest that Deguelin suppresses the invasiveness and metastasis of lung cancer by regulating CD44, MMP-2 and MMP-9 activity.

Studies have demonstrated that he changes in cytogenetic characteristics are responsible for the invasion and metastasis of cancer cells [16]. Because the changes in genetic characteristics have altered the invasion and migration ability so that the cancer cells can penetrate the basilar membrane and extracellular matrix into the blood vessels, and then pierce through the blood vessels after reaching the target organs with blood circulation, and consequently, proliferate in the target organs to form metastatic foci. And the morphologic changes during cell migration involve the rearrangement of actin cytoskeleton, leading to the formation of filopodia and lamellipodia [17]. Polymerization of microfilaments provides power for cell migration and motility. Filopodia may play a main role in the initial stage of tumor cell migration and invasion, mainly including motility, adhesion, nutrition uptake, phagocytosis and support [18]. Lamellipodia is closely related to tumor cell adhesion with the target organs [18, 19]. So regulating the rearrangement of actin cytoskeleton, may lead to cancer cell invasion and metastasis suppression. Our results showed that Deguelin-treated tumor leading
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the rearrangement of actin cytoskeleton and decreasing of filopodia and lamellipodia. Cell spreading is regulated by coordinated changes in integrin-mediated adhesions to ECM and reorganization of the actin cytoskeleton by Rho family GTP-binding proteins [20]. Rac1, one of Rho GTPase family members, is an intracellular molecular switch that transduces signals in various cancers and promotes actin polymerization, inducing the formation of lamellipodia and filopodia [21]. ROCK1 is a serine/threonine kinase that belongs to the Rho family and indirectly diminishes the activity of upstream RhoA by stimulating Rac1 activity [21]. In our study, we found that the expression of Rac1 and Rock1 decreased after Deguelin treated. Our results suggests that Deguelin suppresses cell invasion might through regulating reorganization of the actin cytoskeleton, via Rho GTPase signaling pathways.

In conclusion, Deguelin suppresses human lung cancer cell invasion is mediated by regulating reorganization of the actin cytoskeleton, decreases the expression of CD44, MMP-2 and MMP-9 activity, via Rho GTPase signaling pathways. Our findings provide novel insights into the anti-lung cancer molecular mechanisms of Deguelin, and Deguelin may be a potential therapeutic candidate molecules in the treatment of lung cancer.

Disclosure of conflict of interest

None.

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