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Celastrol Inhibits Proliferation and Metastatic Potential and Induces Apoptosis in ARID1A-Deficient Non-Small Cell Lung Cancer

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Abstract: *Objective:* Non-small cell lung cancer (NSCLC) is still challenging to treat due to its high recurrence rate and high metastasis rate. New effective therapeutic drugs urgently need to be developed. Celastrol is a small-molecule compound from traditional Chinese medicine with broad-spectrum anti-tumor activity, but its mechanism of action on ARID1A KO NSCLC has not been fully studied. This study systematically investigated the in vitro antitumor effects of Celastrol on the human NSCLC cell line A549. *Methods:* The CCK-8 assay was used to assess cell proliferation; wound-healing and Transwell assays evaluated cell migration and invasion, respectively; Annexin V-FITC/PI double staining, combined with flow cytometry, measured cell apoptosis. *Results:* Celastrol significantly inhibited A549 cell proliferation in a dose- and time-dependent manner, reducing cell viability to approximately 40% at 4 μM after 24 h. The wound-healing and Transwell experiments demonstrated that, compared with the control group, triptolide significantly inhibited cell migration and invasion; flow cytometry showed that the total apoptosis rate of the cells increased significantly after triptolide treatment. *Conclusion:* Celastrol effectively inhibits A549 cell proliferation, migration, and invasion, and induces apoptosis in a dose-dependent manner. These findings have confirmed the potential of Celastrol as a new candidate drug for the treatment of ARID1A KO NSCLC, and have provided reliable in vitro evidence for subsequent mechanism studies and in vivo research.

Keywords: NSCLC; ARID1A; Celastrol; Migration; Apoptosis

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1. Introduction

Non-small cell lung cancer (NSCLC) remains one of the most prevalent and lethal malignancies worldwide, accounting for over 85% of all lung cancer cases [1]. According to the latest estimates from the World Health Organization (WHO), approximately 2.5 million new lung cancer cases and nearly 1.8 million deaths occurred globally in 2022. Despite therapeutic advancements, the 5-year survival rate remains approximately 20% [2]. The high mortality rate of NSCLC is attributed to late-stage diagnosis [3,4], resistance to chemotherapy and radiotherapy [5], and significant molecular heterogeneity that complicates treatment selection [6], underscoring the critical need for precision strategies targeting specific molecular subgroups [7].

ARID1A (AT-rich interactive domain 1A) is a core subunit of the SWI/SNF chromatin remodeling complex that maintains epigenetic homeostasis by modulating chromatin accessibility [8]. Recent studies have demonstrated that ARID1A is frequently inactivated in various tumors, which is closely associated with increased malignancy and poor prognosis. In endometrial cancer, ARID1A mutation rates range from 30% to 50% (highest in the endometrioid subtype); these mutations often coexist with POLE ultramutation and deficient mismatch repair (dMMR), leading to a high tumor mutational burden and enhanced immune infiltration [9], which may benefit from immunotherapy despite associations with poor differentiation and recurrence risk. In ovarian clear cell and endometrioid carcinomas, mutation rates reach 40%–60% and frequently co-occur with PI3K pathway mutations (e.g., PIK3CA), promoting metabolic reprogramming and oxidative stress adaptation, thereby driving therapeutic resistance [10]. In NSCLC, ARID1A mutations occur in 5%–10% of cases and are correlated with aggressive phenotypes, poor prognosis, and synergistic interactions with EGFR, KRAS, and Wnt/β-catenin pathways [11].

Natural products have long served as a vital repository for anticancer drug discovery due to their structural diversity and multi-target regulatory properties. Historically, approximately 60% of approved anticancer drugs have been derived, directly or indirectly, from natural sources [12,13]. Among these, Celastrol, a pentacyclic triterpene isolated from the root of *Tripterygium wilfordii* Hook. f. (Thunder God Vine), has garnered significant attention for its potent biological activities [14,15]. Celastrol was identified by leading journals, including Cell, as one of the most promising natural-origin medicinal molecules [16,17]. While initial research focused on its anti-inflammatory and immunosuppressive properties for rheumatoid arthritis, studies over the past decade have revealed broad-spectrum antineoplastic efficacy against various malignancies with relatively low toxicity to normal cells [18].

The present study aims to address a critical knowledge gap by systematically investigating the biological effects of Celastrol on ARID1A-deficient NSCLC cells through in vitro experiments. We sought to determine whether Celastrol inhibits the proliferation of ARID1A-deficient NSCLC cells in a dose- and time-dependent manner, induces programmed cell death by modulating key apoptotic proteins, and significantly impairs their migratory and invasive capabilities at subtoxic concentrations. These findings will not only provide a pharmacological rationale for the application of Celastrol but also offer a potential novel therapeutic strategy for the treatment of ARID1A-deficient NSCLC [19].

2. Materials and methods

2.1. Cell culture

The human non-small cell lung cancer (NSCLC) cell line A549 was purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). To establish the ARID1A-knockout (KO) A549 cell line, CRISPR-Cas9 genome editing was performed to target the ARID1A gene. Celastrol (purity ≥ 98%, HPLC grade) was obtained from Sigma-Aldrich (St. Louis, MO, USA). F12K, fetal bovine serum (FBS), and 0.25% trypsin-EDTA were purchased from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). Penicillin-streptomycin (100 U/mL) and phosphate-buffered saline (PBS) were obtained from HyClone (Logan, UT, USA). The Cell Counting Kit-8 (CCK-8) was provided by Dojindo Molecular Technologies (Kumamoto, Japan). The Annexin V-FITC/PI Apoptosis Detection Kit was purchased from BD Biosciences (San Jose, CA, USA). Matrigel was obtained from Corning (Corning, NY, USA). Dimethyl sulfoxide (DMSO) and crystal violet were purchased from Solarbio Life Sciences (Beijing, China).

ARID1A KO A549 cells were cultured in F12K supplemented with 10% FBS and 1% penicillin-streptomycin in a humidified atmosphere containing 5% CO_2 at 37℃. The culture medium was replaced every 2 days, and cells were passaged using 0.25% trypsin upon reaching 80-90% confluence. Cells in the logarithmic growth phase were used for all subsequent experiments [20,21].

Celastrol was dissolved in DMSO to prepare a stock solution (10 mM) and stored at -20℃ in the dark. For experimental treatments, the stock solution was diluted with culture medium to the final concentrations.

2.2. Cell proliferation assay

Cell viability was assessed using the CCK-8 assay. ARID1A KO A549 cells were seeded into 96-well plates at a density of 5×10^3 cells/well and incubated for 24 h to allow for attachment. Subsequently, cells were treated with various concentrations of Celastrol for 24 and 48 h. At the end of the incubation period, 10 μL of CCK-8 reagent was added to each well, followed by an additional 2-hour incubation at 37 ℃. The absorbance (OD) was measured at 450 nm using a microplate reader. The relative cell proliferation rate was calculated as follows:

$$\text{Proliferation Rate (\%)} = (OD_{treated}/OD_{control}) \times 100\%$$

2.3. Wound-healing assay

To evaluate cell migration, ARID1A KO A549 cells were seeded in 6-well plates 5×10^5 cells/well) and cultured until reaching approximately 90% confluence. A straight scratch was created in the monolayer using a sterile 200 μL pipette tip. After washing three times with PBS to remove cellular debris, the cells were incubated in serum-free medium containing different concentrations of Celastrol. Images of the wounds were captured at 0 and 24 h using an inverted microscope. The wound area was quantified using ImageJ software, and the wound healing rate was calculated as:

$$\text{Wound Healing Rate (\%)} = (Area_{0h} - Area_{th})/Area_{0h} \times 100\%$$

2.4. Transwell invasion assays

For the invasion assay, the upper chambers were pre-coated with 50 μL of diluted Matrigel (1:8 ratio with serum-free medium) and incubated at 37 ℃ for 30 min to form a basement membrane. The remaining steps were identical to the migration assay. After 24 h of incubation, the cells on the upper surface were removed with cotton swabs. The migrated or invaded cells on the lower surface were fixed with 4% paraformaldehyde for 30 min and stained with 0.1% crystal violet for 20 min. Five random fields were selected for counting under a microscope to quantify cellular translocation.

2.5. Flow cytometry analysis of apoptosis

ARID1A KO A549 cells were seeded into 6-well plates 5×10^5 cells/well) and treated with Celastrol for 24 h. Following treatment, cells were harvested by trypsinization (without EDTA), washed twice with ice-cold PBS, and resuspended in 195 μL of Annexin V-FITC binding buffer. The cells were then incubated with 5 μL of Annexin V-FITC for 10 min, followed by 10 μL of Propidium Iodide (PI) for 5 min in the dark at room temperature. Apoptotic rates were immediately analyzed using a NovoExpress Flow Cytometer. Data were processed using FlowJo software (v10.8), and the apoptosis rate was defined as the sum of early and late apoptotic populations.

2.6. Statistical analysis

All experimental data are expressed as the mean ± standard deviation (SD) from at least three independent experiments. Comparisons between multiple groups were conducted using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. Comparisons between two groups were performed using Student's t-test. A P-value < 0.05 was considered statistically significant ($^*P < 0.05$, $^{**}P < 0.01$). Statistical analyses and graphical representations were generated using GraphPad Prism 9.0.

3. Results

3.1. Celastrol inhibits the proliferation of ARID1A KO NSCLC

To evaluate the inhibitory effect of Celastrol on the growth of ARID1A KO NSCLC cells, A549 cells were treated with gradient concentrations of Celastrol for 24 h. The CCK-8 assay results revealed that Celastrol significantly suppressed A549 cell proliferation compared with the blank and vehicle control groups ($P < 0.05$) (**Figure 1**). The inhibitory effect became more pronounced with increasing concentrations of Celastrol and prolonged incubation time, demonstrating a clear

dose- and time-dependent relationship. Specifically, after 24 h of treatment with 4 μM Celastrol, cell viability decreased to approximately 40% of the control group level.

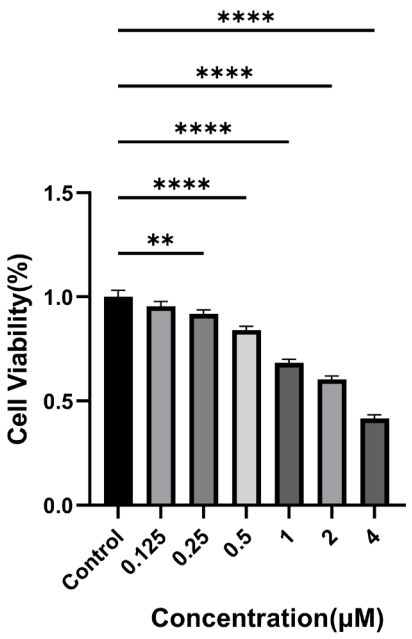

Figure 1. Celastrol suppresses the proliferation of ARID1A KO A549 cells.The relative cell proliferation rate of ARID1A KO A549 cells after being treated with different concentrations of Celastrol for 24 hours. Data are presented as mean ± SD (n = 3). $^{**}P < 0.01$ and $^{****}P < 0.0001$ vs. control group.

3.2. Celastrol impairs the migratory capacity of ARID1A KO NSCLC

The effect of Celastrol on the lateral migration of A549 cells was assessed using a wound-healing assay. At 0 h, the initial wound widths were uniform across all experimental groups ($P > 0.05$). After 24 h of incubation, the wound-healing rates in the Celastrol-treated groups were significantly lower than those in the control groups ($P < 0.01$) (**Figure 2**). These findings suggest that Celastrol effectively impairs the horizontal migratory ability of NSCLC cells in a dose-dependent manner.

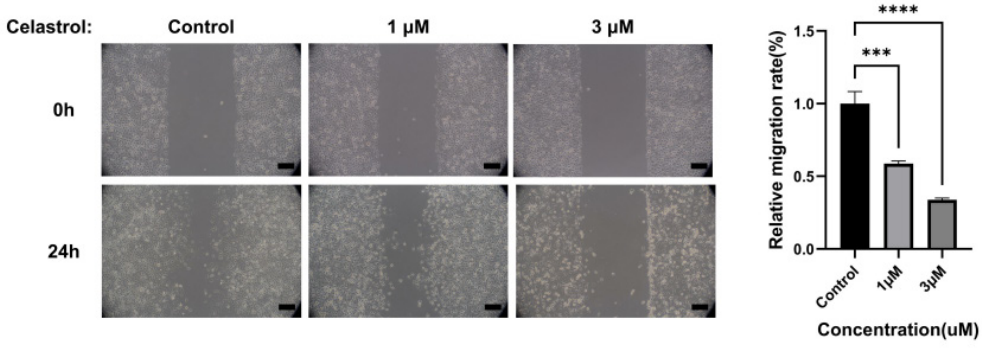

Figure 2. Celastrol inhibits the migration of ARID1A KO A549 cells in wound-healing assays.
(A) Representative microscopic images of the wound area at 0 and 24 h post-scratching (Scale bar = 100 μm). (B) Quantitative analysis of the wound healing rate at 24 h. Data are expressed as mean ± SD (n=3). $^{****}P < 0.001$,$^{****}P < 0.0001$ vs. control group.

3.3. Celastrol suppresses the invasion potential of ARID1A KO NSCLC

To further investigate the invasive potential of NSCLC cells, Transwell assays were performed. The results showed that in the Matrigel invasion assay, Celastrol treatment led to a dose-dependent decrease in the number of invaded cells (**Figure 3**). Quantitative analysis indicated that Celastrol effectively suppresses the metastatic potential of NSCLC cells by inhibiting both migration and invasion.

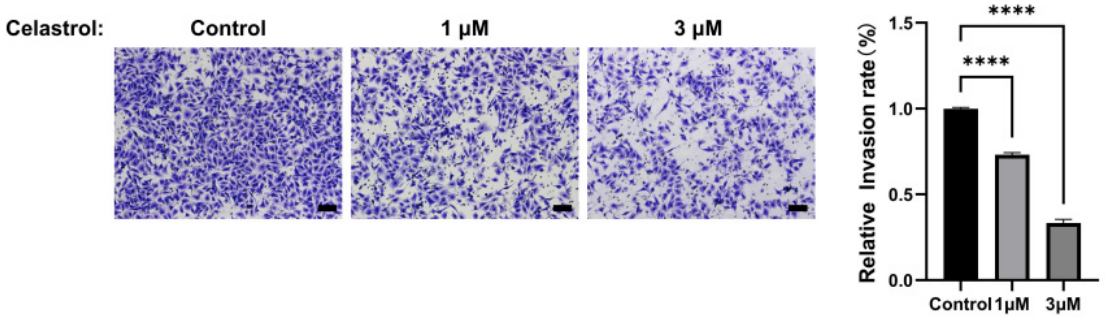

Figure 3. Celastrol suppresses ARID1A KO A549 cell migration and invasion in Transwell assays. (A) Representative images of invaded cells stained with crystal violet (Scale bar = 100 μm). (B) Statistical quantification of the number of migrated and invaded cells per field. Data are shown as mean ± SD (n=3). ****$P < 0.0001$ vs. control group.

3.4. Celastrol induces apoptosis in ARID1A KO NSCLC

To determine whether the growth inhibition was associated with programmed cell death, Annexin V-FITC/PI double-staining followed by flow cytometry was used to detect apoptosis. As shown in **Figure 4**, the apoptosis rates in the control and vehicle groups remained at baseline levels (approximately 5–7%). However, treatment with Celastrol for 24 h led to a significant, dose-dependent increase in the total apoptotic rate (including both early and late apoptosis). At a concentration of 10 μM, the total percentage of apoptotic cells increased to approximately 45.2% ($P < 0.01$). These results confirm that Celastrol exerts its anti-tumor activity in NSCLC cells, at least in part, by inducing apoptosis.

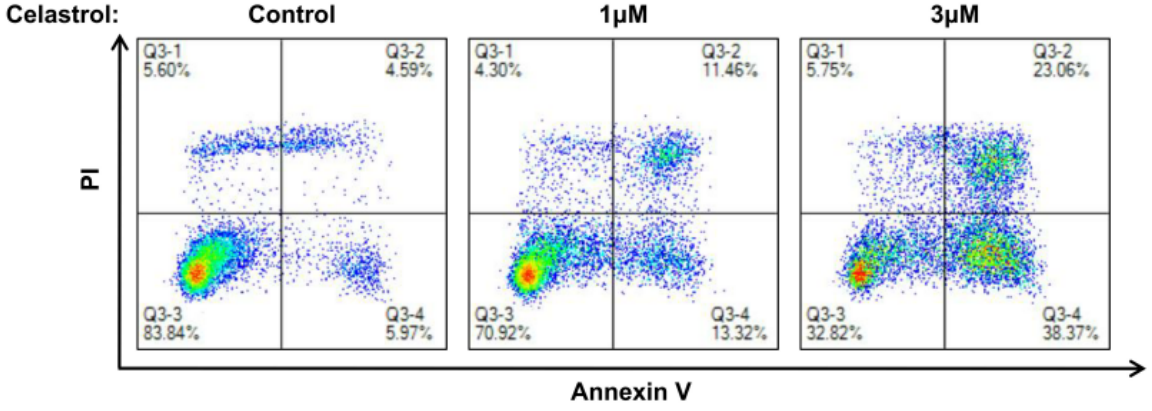

Figure 4. Celastrol promotes apoptosis in NSCLC. (A) Flow cytometric analysis of ARID1A KO A549 cell apoptosis using Annexin V-FITC/PI double staining after 24 h of treatment.

4. Discussion

This study systematically evaluated the antitumor effects of Celastrol on the ARID1A KO NSCLC cell line A549. Our

results demonstrated that Celastrol significantly inhibited cell proliferation, suppressed migratory and invasive capacities, and induced apoptosis in ARID1A KO A549 cells. The CCK-8 assay confirmed that the growth-inhibitory effect was dose- and time-dependent. These multifaceted inhibitory effects suggest that Celastrol interferes with multiple hallmark pathways involved in ARID1A KO A549 progression.

Our findings are consistent with the broader pharmacological profile of Celastrol, reinforcing its reputation as a potent antitumor agent. For instance, Celastrol has been reported to induce ROS-mediated apoptosis and cell cycle arrest in lung and liver cancer models, which parallels the pro-apoptotic trends observed in our A549 model. However, compared to malignancies such as gastric or breast cancer, studies specifically investigating the effects of Celastrol on ARID1A KO NSCLC remain limited.

This study provides evidence that Celastrol retains its potent antitumor properties in this particular epithelial malignancy, thereby supporting its potential as a broad-spectrum natural anticancer compound. Moreover, it addresses a specific gap in ARID1A NSCLC research by systematically characterizing the phenotypic responses to Celastrol treatment.

From a theoretical standpoint, this work contributes to a more comprehensive understanding of Celastrol‘s pharmacological spectrum. Clinically, these findings highlight the potential of Celastrol as a candidate for further development, either as a standalone therapeutic agent or in combination with existing treatments.

Despite these promising results, several limitations should be acknowledged. First, this study was restricted to in vitro experiments using a single NSCLC cell line (A549). Future investigations should incorporate a broader panel of NSCLC cell lines and, more importantly, employ in vivo xenograft models to evaluate the safety and efficacy of Celastrol in a complex physiological environment. Second, although significant phenotypic changes were observed, the precise upstream molecular targets and specific signaling pathways through which Celastrol exerts its inhibitory effects in ARI1DA KO NSCLC cells remain to be elucidated. Further mechanistic studies are warranted to clarify these pathways and to identify potential biomarkers for patient stratification.

5. Conclusion

In summary, this study demonstrates that Celastrol exerts potent antitumor activity against ARID1A KO Non-small cell lung cancer (NSCLC) cells in vitro. Our findings indicate that Celastrol significantly inhibits the proliferation of ARID1A KO NSCLC cells and induces apoptosis in a dose-dependent manner. Furthermore, Celastrol effectively suppresses the metastatic potential of ARID1A KO NSCLC cells by impairing their migratory and invasive capacities. These results further support the potential of Celastrol as a promising therapeutic candidate for the treatment of ARID1A KO Non-small cell lung cancer.

Disclosure statement

The author declares no conflict of interest.

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