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PRECLINICAL STUDIES

# A high-throughput drug screen identifies auranofin as a potential sensitizer of cisplatin in small cell lung cancer

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## Summary

Small cell lung cancer (SCLC) is a highly lethal malignancy with the 5-year survival rate of less than 7%. Chemotherapy-resistance is a major challenge for SCLC treatment in clinic. In the study, we developed a high-throughput drug screen strategy to identify new drugs that can enhance the sensitivity of chemo-drug cisplatin in SCLC. This screen identified auranofin, a US Food and Drug Administration (FDA)-approved drug used therapeutically for rheumatoid arthritis, as a sensitizer of cisplatin. Further study validated that auranofin synergistically enhanced the anti-tumor activity of cisplatin in chemo-resistant SCLC cells, which was accompanied by the enhanced induction of cell cycle arrest and apoptosis. The synergistic action of auranofin and cisplatin was through ROS overproduction, thereby leading to mitochondrial dysfunction and DNA damage. Furthermore, in vivo study demonstrated that the combination treatment of auranofin and cisplatin dramatically inhibited tumor growth in SCLC. Therefore, our study provides a rational basis for further clinical study to test whether auranofin could enhance the sensitivity of cisplatin-based therapy in SCLC patients.

**Keywords** Small cell lung cancer · Ciplatin · Auranofin · ROS · DNA damage

### Abbreviations

| | |
|---|---|
| SCLC | Small cell lung cancer |
| NSCLC | Non-small cell lung cancer |
| FDA | Food and Drug Administration |
| MMP | Mitochondrial membrane potential |
| CI | Combination index |

## Introduction

Lung cancer has been one of the commonly diagnosed causes of cancer deaths worldwide [1]. Lung cancer includes two major histological subtypes: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) [2]. SCLC is a highly malignant neuroendocrine tumor, which accounts for 15%–20% of total lung cancer cases [3]. Platinum-based chemotherapy (cisplatin and carboplatin) combined with etoposide is the standard first line treatment for SCLC [4]. In spite of the initial high response rate, the major issue for SCLC treatment is almost universal disease recurrence and progression within 3–6 months due to chemo-resistance. Therefore, the development of novel and effective drugs to overcome SCLC chemotherapy-resistance is urgently needed [5].

In the study, we developed a high-throughput drug screen strategy to identify new drugs that can synergistically enhance anti-tumor efficiency of cisplatin in chemo-resistant SCLC cells. An FDA-approved drug library of 1092 compounds was screened when combined with cisplatin in chemo-resistant SCLC cells. The approved drugs have been intensively investigated for safety and pharmacokinetics. Therefore, repurposing approved drugs will increase the speed

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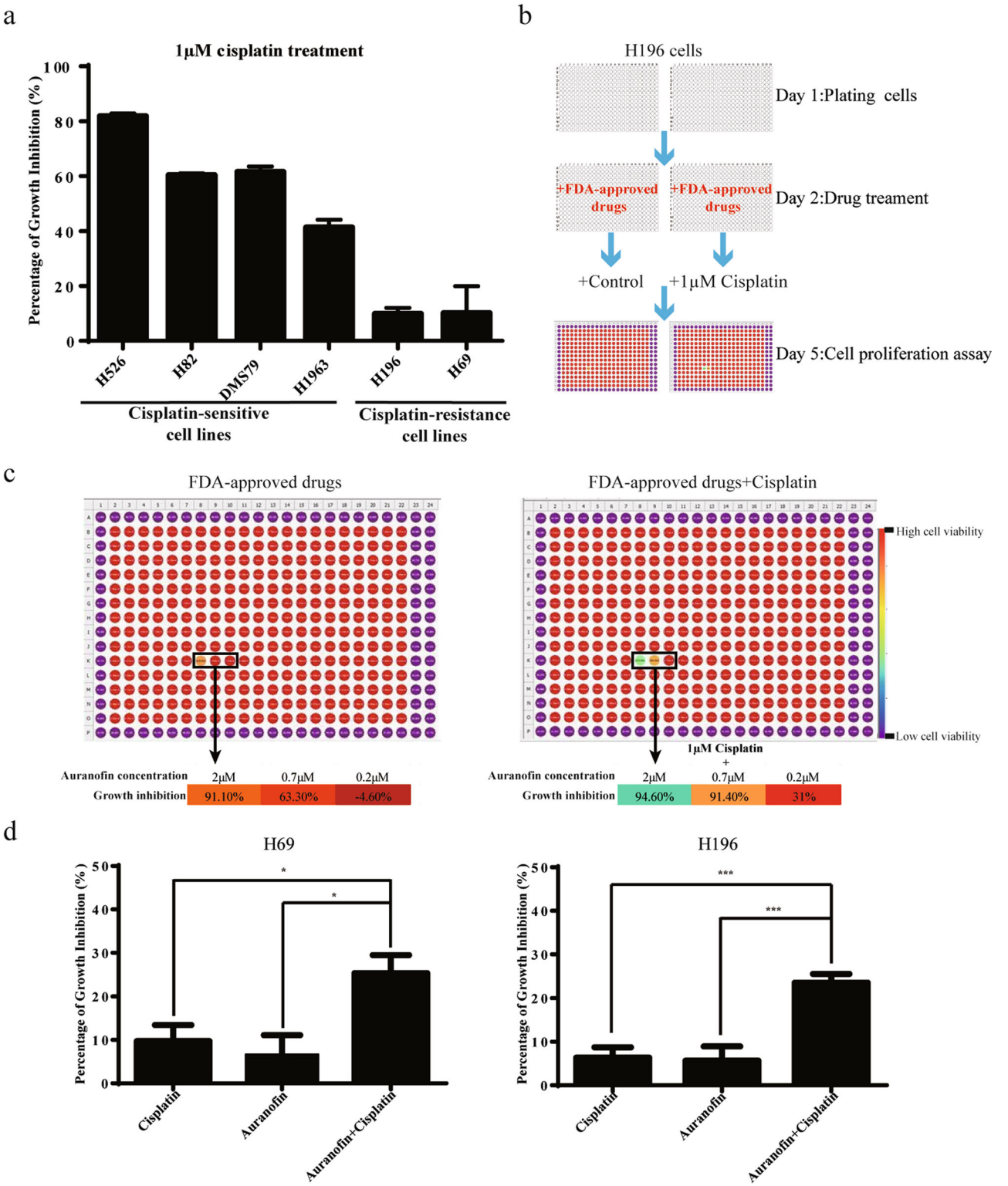



**Fig. 1 High-throughput drug screen identifies auranofin that enhances the sensitivity of SCLC cells to cisplatin a** The anti-tumor activity of cisplatin in a panel of SCLC cell lines. SCLC cell lines(H526、H82、DMS79、H1963、H196、H69)were treated with 1 μM cisplatin for 72 h. Growth inhibition was determined by CellTiter-Glo Luminescent assay. **b** A schematic model illustrating a combinational strategy of high-throughput drug screen to identify drugs that synergize with cisplatin. **c** The plate map of high-throughput drug screen that identified auranofin, which can enhance the anti-proliferative activity of cisplatin in H196 cells. The growth inhibition of H196 cells by auranofin alone or combined with 1 μM cisplatin was indicated. (d) The validation of drug screen. H69 and H196 cells were treated with DMSO control, auranofin (250 nM), cisplatin (1 μM) or the combination of auranofin and cisplatin for 72 h. After treatment, growth inhibition was determined by CellTiter-Glo Luminescent assay

**Table 1** Combination index (CI) of Auranofin and Cisplatin in H69 cells

| Auranofin dose (nM) $D_1$ | Cisplatin dose (nM) $D_2$ | Mean growth inhibition (%) X | Dose of Auranofin alone with same inhibition (nM) $Dx_1$ | Dose of Cisplatin alone with same inhibition (nM) $Dx_2$ | CI |
|---|---|---|---|---|---|
| 250 | 500 | 24 | 630 | 4365 | 0.511 |
| 250 | 1000 | 29 | 398 | 6309 | 0.786 |
| 500 | 500 | 68 | 1000 | 4786 | 0.604 |
| 500 | 1000 | 78 | 794 | 6309 | 0.789 |
| 1000 | 500 | 91 | 1258 | 14454 | 0.830 |

of drug development for chemo-sensitizer in SCLC. Using the combinational high-throughput drug screen, we discovered auranofin that synergized with cisplatin in SCLC.

Auranofin is an alkylphosphine gold coordination complex clinically used in the treatment of rheumatoid arthritis [6]. Auranofin has been shown to have an excellent safety profile in human, and its use is well tolerated in patients with rheumatoid arthritis [7, 8]. A study of high-throughput drug screen has identified auranofin, which was highly effective against *Entamoeba histolytica* infection, leading to its quick FDA approval for the treatment of amebiasis [9].

In the study, we found that auranofin was able to synergistically enhance anti-tumor activity of cisplatin in SCLC. The synergistic action of auranofin and cisplatin was through ROS overproduction, thereby leading to mitochondrial dysfunction and DNA damage. Our study suggests that auranofin is a promising chemo-sensitizer of cisplatin-based therapy in SCLC.

## Materials and methods

### Cell lines

The human SCLC cell lines, H69 and H196 were maintained in RPMI-1640 media (Corning, Cellgro, Manassas, VA, USA) supplemented with 10% fetal bovine serum (ExCell Bio, Shanghai, China) and 1% penicillin/streptomycin (Gibco, Life Technologies, Carlsbad, CA, USA) in a humidified incubator at 37 °C in 5% $CO_2$.

### Materials

Cisplatin was purchased from Selleck chemical (Shanghai, China) and stock solutions were prepared in PBS at a concentration of 5 mM. Auranofin was purchased from MCE chemical (Shanghai, China) and stock solutions were prepared in DMSO (Sigma-Aldrich, Saint Louis, MO, USA) at a concentration of 10 mM. NAC was purchased from MCE chemical (Shanghai, China) and stock solutions were prepared in sterile water. Antibodies against PARP and γH2AX were from Cell Signaling Technology, Danvers, MA, USA. Actin antibody was from TransBionovo, Beijing, China.

### High-throughput drug screen

High-throughput drug screen was conducted using a JANUS automated liquid handling workstation (PerkinElmer, Waltham, MA, USA). H196 cells (1000 cells/well in 384 well plate) were treated with FDA-approved drugs at 3 concentrations (0.2 μM/0.7 μM/2 μM) in the absence or presence of 1 μM cisplatin for 72 h. After treatment, cell proliferation was measured by CellTiter-Glo Luminescent assay (Promega, Madison, WI, USA).

**Table 2** Combination index (CI) of Auranofin and Cisplatin in H196 cells

| Auranofin dose (nM) $D_1$ | Cisplatin dose (nM) $D_2$ | Mean growth inhibition (%) X | Dose of Auranofin alone with same inhibition (nM) $Dx_1$ | Dose of Cisplatin alone with same inhibition (nM) $Dx_2$ | CI |
|---|---|---|---|---|---|
| 500 | 500 | 18 | 812 | 2290 | 0.834 |
| 500 | 1000 | 40 | 741 | 5248 | 0.866 |
| 500 | 2000 | 86 | 1095 | 13182 | 0.414 |
| 1000 | 1000 | 69.6 | 2238 | 9120 | 0.557 |
| 1000 | 2000 | 90 | 2041 | 19054 | 0.595 |

## Cell viability assay

H69 and H196 cells were seeded into 96-well plates with 3000 cells per well, and then incubated with drugs or DMSO control for 72 h. Cell viability was determined by CellTiter-Glo Luminescent assay (Promega, Madison, WI, USA) and normalized to DMSO treatment.

## Cell cycle analysis

After treatment with drugs or DMSO control, cells were fixed in 70% cold ethanol by dropwise addition, and then stained with PI/RNase staining buffer (BD Pharmingen, San Diago, CA, USA). Flow cytometry was performed using a FACS Calibur (CytExpert, Beckman Coulter, Brea, CA, USA), and results were analyzed by FlowJo V10 software (FlowJo LLC, Ashland, Oregon, USA).

## Apoptotic assay

After cells were treated with drugs or DMSO control, apoptosis was analyzed with FITC Annexin V and PI staining using a FITC Annexin V Apoptosis Detection Kit (BD Pharmingen, San Diago, CA, USA). Apoptotic cells were determined by Flow cytometry using a FACS Calibur (CytExpert, Beckman Coulter, Brea, CA, USA), and results were analyzed by FlowJo V10 software (FlowJo LLC, Ashland, Oregon, USA).

## Immunofluorescence staining

SCLC cells ($1 \times 10^6$ cells per well in six-well plates) were treated with drugs or vehicle control (DMSO). After treatment, cells were washed with PBS, fixed with freshly prepared 4% paraformaldehyde, and then permeabilized with 0.01% Triton X-100 in TBS. After blocked with pre-cooling AbDil-TX (TBS-Tx containing 2% bovine serum albumin and 0.05% sodium azide) for 2 h at room temperature, the samples were incubated with primary rabbit anti-phospho-histone H2AX (Ser139) antibody (1:200) at 4 °C overnight followed by the anti-rabbit-FITC secondary antibody (Thermo Fisher Scientific, Waltham, MA, USA) for 1 h at room temperature. After washing, samples were stained with DAPI for 1 min, mounted, and examined by fluorescence microscopy (Leica, Wetzlar, Germany).

## Measurement of ROS generation

H69 and H196 cells were seeded in six-well plates at $1 \times 10^6$ cells per well overnight, and treated with DMSO control, auranofin (1 μM), cisplatin (1 μM), or the combination of auranofin and cisplatin for 4 h. Cells were stained with 10 μM DCFH-DA (Beyotime Biotech, Shanghai, China) at 37 °C for 30 min, and washed with PBS. Then H69 cells (suspension cells) were collected and the fluorescence was analyzed using a FACS Calibur (CytExpert, Beckman Coulter, Brea, CA, USA). The fluorescence of H196 cells (adherent cells) was examined by fluorescence microscopy (Leica, Wetzlar, Germany).

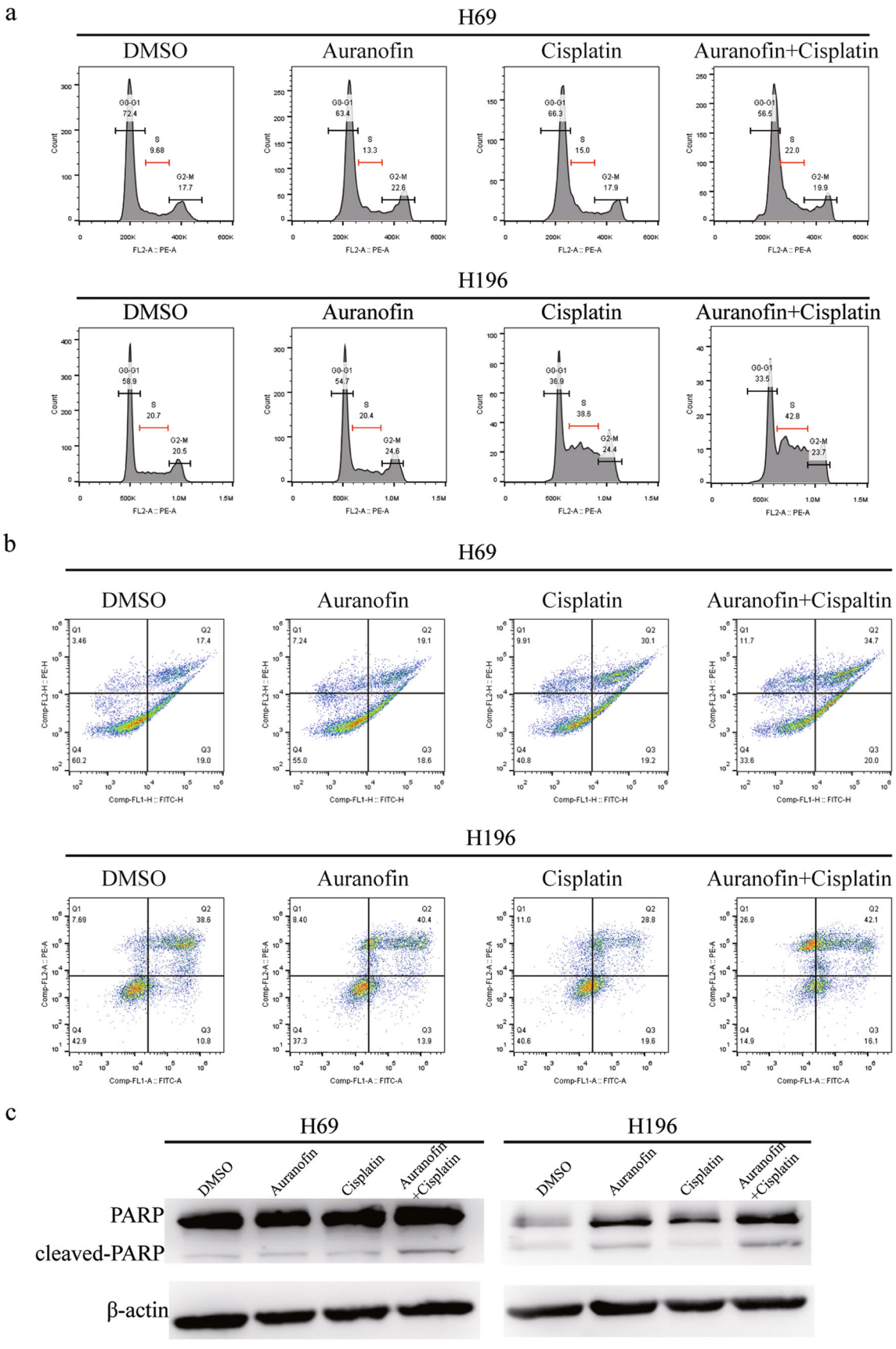
**Fig. 2 Auranofin enhances cisplatin-induced S-phase cell cycle arrest and apoptosis a** Auranofin and cisplatin co-treatment induced stronger cell cycle arrest in S phase. H69 and H196 cells were treated with DMSO control, auranofin (250 nM), cisplatin (1 μM), or the combination of auranofin and cisplatin for 24 h. After treatment, flow cytometry was performed. **b** Auranofin and cisplatin co-treatment induced stronger apoptosis as indicated by Annexin V staining. H69 and H196 cells were treated with DMSO control, auranofin (250 nM), cisplatin (1 μM), or the combination of auranofin and cisplatin for 48 h. After treatment, Annexin V apoptotic assay was performed by flow cytometry. The percentage of apoptotic cells (Annexin V positive) was shown in Q2 + Q3. **c** Auranofin and cisplatin co-treatment induced stronger apoptosis as indicated by PARP cleavage. H69 and H196 cells were treated with DMSO control, auranofin (250 nM), cisplatin (1 μM), or the combination of auranofin and cisplatin for 24 h. PARP and cleaved PARP were detected by western blot. β-Actin was used as loading control

## Evaluation of MMP

MMP was examined by fluorescence microscopy using JC-1 (Beyotime Biotech, Shanghai, China) as a probe. Cells were treated with drugs for 24 h and stained with JC-1 at 37 °C for 30 min and then H196 (adherent cells) cells were viewed under the fluorescence microscopy to analyzed the shift of JC-1 aggregate to JC-1 monomer (Leica, Wetzlar, Germany).

## Xenograft experiments

Animal experiments were carried out according to a protocol approved by Institutional Animal Care and Use Committee of Hefei Institutes of Physical Science. Five-week-old athymic nude mice were injected subcutaneously in dorsal flank, with a 100 μL suspension of $2 \times 10^6$ H69 cells in an equal volume of Matrigel (BD Biosciences, Franklin, NJ, USA). When tumors grew to 4 to 5 mm in diameter, the mice were randomly divided into four groups (5 mice/group), and then treated by intraperitoneal injection with DMSO control, auranofin (10 mg/kg), cisplatin (2 mg/kg) and the combination of auranofin (10 mg/kg) with cisplatin (2 mg/kg) every two days. The tumor size was monitored by caliper measurements and calculated by the formula: Volume = (length ×width × width)/2.


a
H69
DMSO
Auranofin
Cisplatin
Auranofin+Cisplatin
G0-G1 72.4
S 9.68
G2-M 17.7
G0-G1 63.4
S 13.3
G2-M 22.6
G0-G1 66.3
S 15.0
G2-M 17.9
G0-G1 56.5
S 22.0
G2-M 19.9
H196
G0-G1 58.9
S 20.7
G2-M 20.5
G0-G1 54.7
S 20.4
G2-M 24.6
G0-G1 36.9
S 38.6
G2-M 24.4
G0-G1 33.5
S 42.8
G2-M 23.7
Count
FL2-A :: PE-A
b
H69
DMSO
Auranofin
Cisplatin
Auranofin+Cispaltin
Q1 3.46
Q2 17.4
Q4 60.2
Q3 19.0
Q1 7.24
Q2 19.1
Q4 55.0
Q3 18.6
Q1 9.91
Q2 30.1
Q4 40.8
Q3 19.2
Q1 11.7
Q2 34.7
Q4 33.6
Q3 20.0
Comp-FL2-H :: PE-H
Comp-FL1-H :: FITC-H
H196
DMSO
Auranofin
Cisplatin
Auranofin+Cisplatin
Q1 7.69
Q2 38.6
Q4 42.9
Q3 10.8
Q1 8.40
Q2 40.4
Q4 37.3
Q3 13.9
Q1 11.0
Q2 28.8
Q4 40.6
Q3 19.6
Q1 26.9
Q2 42.1
Q4 14.9
Q3 16.1
Comp-FL2-A :: PE-A
Comp-FL1-A :: FITC-A
c
H69
H196
DMSO
Auranofin
Cisplatin
Auranofin +Cisplatin
PARP
cleaved-PARP
β-actin

### H&E staining and immunochemistry

Tumors were harvested from euthanized mice, fixed in 4% paraformaldehyde for 24 h and embedded in paraffin wax. H&E Staining and Ki67 immunochemistry were performed as previously described [10].

### Assessment of drug synergy

Drug synergy was determined quantitatively using the combination index (CI) method of Chou and Talalay [11]. CI was calculated using the formula: $CI = D_1/DX_1 + D_2/DX_2$. $DX_1$ and $DX_2$ are drug dose in which the growth inhibition rate reaches a specific value when the two drugs are used alone, and $D_1$ and $D_2$ are the doses of the two drugs when the two drugs are combined to achieve the same growth inhibition rate. $CI < 1$ indicates synergism, whereas $CI > 1$ indicates antagonism.

### Statistical analysis

All data were analyzed using PRISM5 Software (GraphPad Software, Inc., La Jolla, CA, USA). Statistical analysis was performed using an unpaired *t*-test. Results were considered as statistically significant when $P < 0.05$.

## Results

### High-throughput drug screen identifies auranofin that enhances the sensitivity of SCLC cells to cisplatin

To improve the therapeutic outcome of cisplatin for SCLC, we developed a combinational drug screen strategy to identify drugs that enhance the anti-tumor activity of cisplatin in SCLC cells. Firstly, we tested the sensitivity of various SCLC cell lines (H526, H82, DMS79, H69, H196 and H1963) to cisplatin. As indicated in Fig. 1a, H196 and H69 cell lines were resistant to cisplatin, while other cell lines H526, H82, DMS79 and H1963 were relatively sensitive. Therefore, we selected H196 cell line for the high-throughput drug screen. FDA-approved drug library was screened when combined with cisplatin treatment in H196 cells. The growth inhibition of H196 cells induced by FDA-approved drugs with or without cisplatin was determined by CellTiter-Glo Luminescent assay (Fig. 1b). The screen of 1092 FDA-approved drugs identified auranofin, which was able to enhance the anti-proliferative activity of cisplatin in H196 cell (Fig. 1c). We further validated the increased anti-growth activity of cisplatin when combined with auranofin in H69 and H196 cells (Fig. 1d). Next, we calculated the combination index (CI) to examine whether the combination of auranofin and cisplatin exerted synergistic anti-proliferative activity in SCLC. As indicated in Tables 1 and 2, the combination of auranofin and cisplatin showed synergistic anti-tumor effect as the values of CI in both H69 and H196 cells were less than one. These results indicate that auranofin synergistically enhances anti-tumor activity of cisplatin in SCLC.

### Auranofin enhances cisplatin-induced S-phase cell cycle arrest and apoptosis

To examine the synergistic mechanism of auranofin and cisplatin combination, the effect of their combination on cell cycle and apoptosis was studied in SCLC. Cell cycle analysis indicated that auranofin enhanced cisplatin-induced S-phase cell cycle arrest in cisplatin-resistant H69 and H196 cells (Fig. 2a). Similarly, Annexin V apoptotic assay indicated that auranofin enhanced cisplatin-induced apoptosis in H69 and H196 cells (Fig. 2b). Western blot showed that the combination of auranofin and cisplatin induced more PARP-cleavage (apoptotic marker), when compared with auranofin or cisplatin single treatment in H69 and H196 cells (Fig. 2c). The data suggests that auranofin increases the anti-tumor activity of cisplatin through the enhanced cell cycle arrest and apoptosis in SCLC.

### Auranofin enhances cisplatin-induced cell death through ROS overproduction

Previous study has indicated that auranofin induced ROS production through inhibiting thioredoxin reductase (TrxR) activity [12]. Therefore, we measured intracellular ROS generation when H196 and H69 cells were treated with DMSO control, auranofin, cisplatin and their combination. As shown in Fig. 3a and b, auranofin treatment caused the increase of intracellular ROS level, while the combination of auranofin and cisplatin induced more ROS production in H69 and H196 cells. In order to demonstrate that ROS overproduction was involved in the enhanced cell death induced by the combination of auranofin and cisplatin. NAC, a thiol-reducing antioxidant agent was used to scavenge ROS. Pretreatment of H69 and H196 cells with 5 mM NAC for 1 h effectively attenuated ROS overproduction caused by auranofin and cisplatin co-treatment (Fig. 3c). Furthermore, the cell death induced by auranofin and cisplatin co-treatment was completely rescued by NAC pretreatment in H69 and H196 cells (Fig. 3d). Our results indicate that the combination of auranofin and cisplatin induces ROS accumulation, thereby leading to the enhanced SCLC cell death.

### The combination of auranofin and cisplatin induces ROS-mediated mitochondrial dysfunction and DNA damage

The mitochondrion is the major source of cellular ROS, and ROS overproduction can directly cause the loss of mitochondrial membrane potential (MMP), resulting in

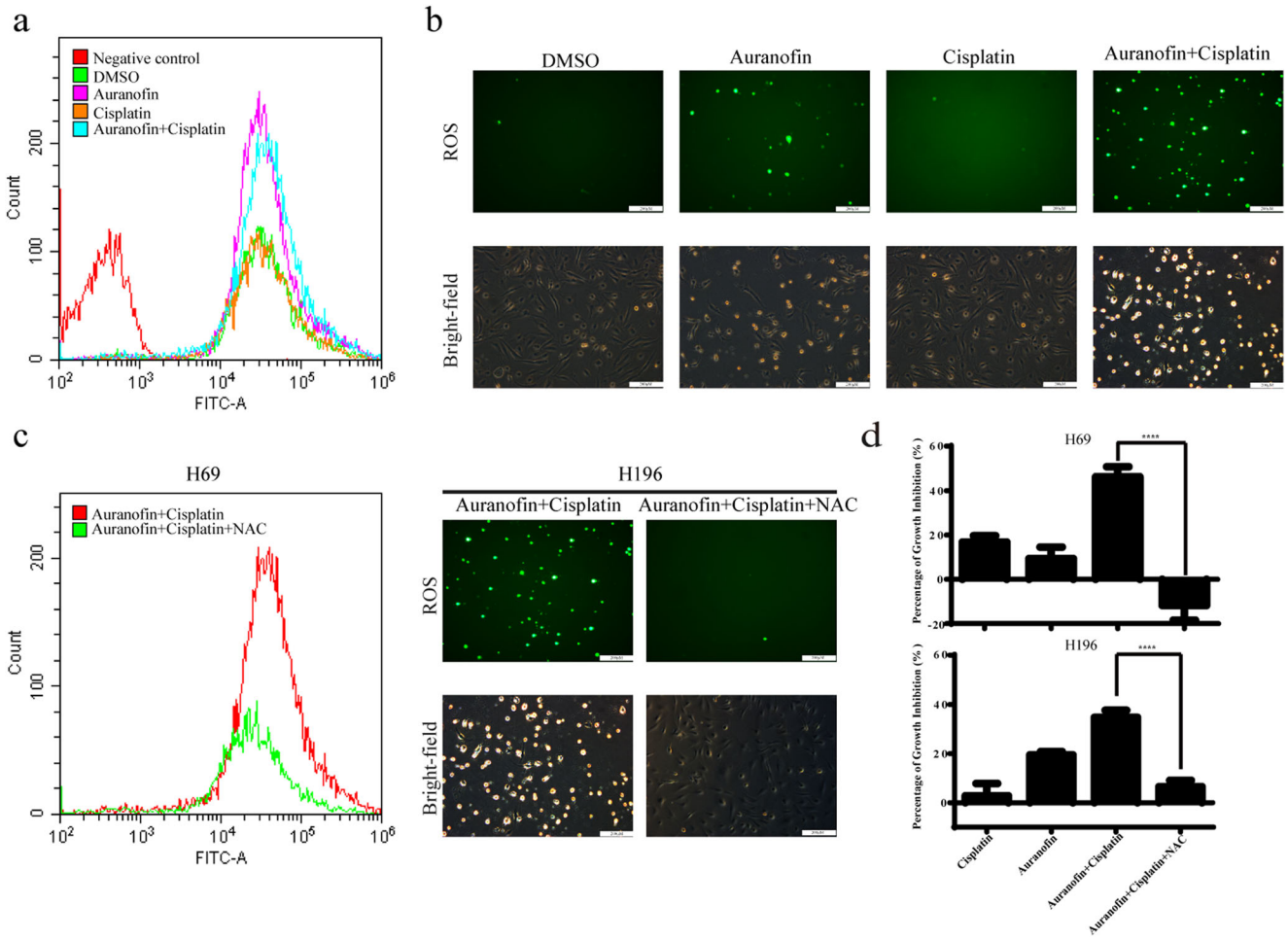



**Fig. 3 Auranofin enhances cisplatin-induced cell death through ROS overproduction a** The combination of auranofin and cisplatin induced more intracellular ROS generation detected by flow cytometry. H69 cells were treated with DMSO control, auranofin (1 μM), cisplatin (1 μM), or the combination of auranofin and cisplatin for 4 h. After treatment, cells were stained with DCFH-DA, and then ROS was detected by flow cytometry. The peak (blue color) of auranofin and cisplatin co-treatment was shifted to the right. **b** The combination of auranofin and cisplatin induced more intracellular ROS generation detected by fluorescence microscopy. H196 cells were treated with DMSO control, auranofin (1 μM), cisplatin (1 μM), or the combination of auranofin and cisplatin for 4 h. After treatment, cells were stained with DCFH-DA, and then ROS (green fluorescence) were examined by fluorescence microscopy. **c** ROS scavenger, NAC completely rescued the overproduction of ROS induced by the combined treatment of auranofin and cisplatin in SCLC. H69 and H196 cells were pretreated with NAC for 2 h. And then cells were treated with DMSO control, auranofin (1 μM), cisplatin (1 μM), or the combination of auranofin and cisplatin for 4 h. After treatment, cells were stained with DCFH-DA, and then ROS was detected by flow cytometry (H69 cells) or fluorescence microscopy (H196 cells). **d** NAC completely rescued the growth inhibition induced by the combined treatment of auranofin and cisplatin in SCLC cells. H69 and H196 cells were pretreated with NAC for 2 h. And then cells were treated with DMSO control, auranofin, cisplatin, or the combination of auranofin and cisplatin for 72 h. After treatment, growth inhibition was determined by CellTiter-Glo Luminescent assay

mitochondrial dysfunction [13]. Therefore, we next examined whether auranofin and cisplatin co-treatment induced mitochondrial dysfunction. As shown by JC-1 (a fluorescent probe of MMP**)** staining, the combined treatment dramatically decreased the integrity of MMP as demonstrated by the shift of JC-1 aggregates (red fluorescence) to JC-1 monomer (green fluorescence) (Fig. 4a). Excessive generation of intracellular ROS is a main cause of DNA damage, which induces cell cycle arrest and apoptosis. In Fig. 4b, the combination of auranofin and cisplatin induced stronger DNA damage, as indicated by the induction of γH2AX, a typical marker of double-strand DNA breaks. Our results demonstrate that the combination of auranofin and cisplatin induces mitochondrial dysfunction and DNA damage in SCLC.

## Auranofin/cisplatin combination potently reduces SCLC tumor growth in vivo

To further evaluate the anti-tumor efficiency of the combination of auranofin and cisplatin in vivo, immuno-deficient nude mice model bearing xenografted H69 SCLC cells by subcutaneous injection were established. The mice with xenografted

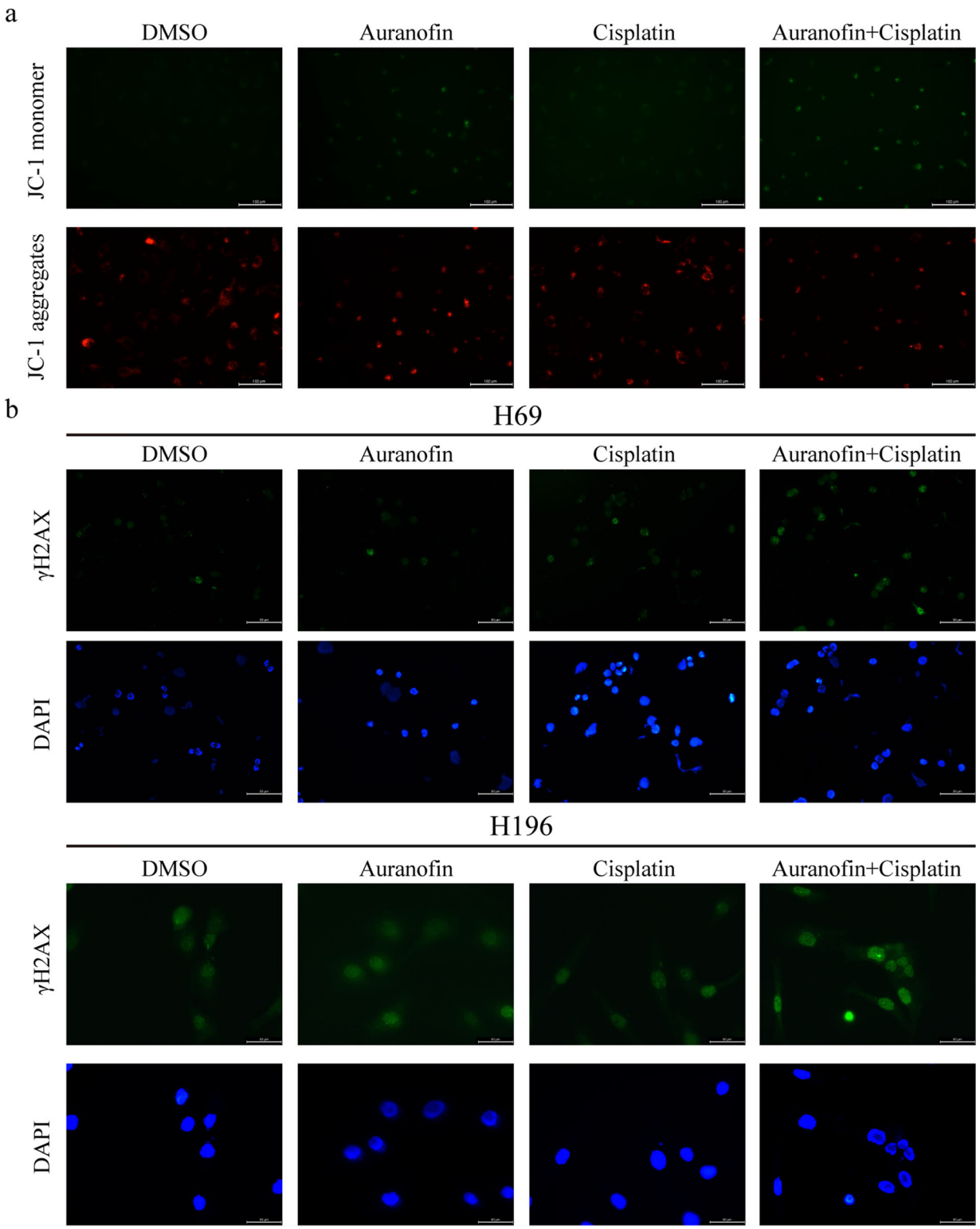



**Fig. 4 The combination of auranofin and cisplatin induces ROS-mediated mitochondrial dysfunction and DNA damage a** Auranofin and cisplatin co-treatment obviously decreased the MMP in SCLC. H196 cells were treated with DMSO control, auranofin (1 μM), cisplatin (1 μM), or the combination of auranofin and cisplatin for 24 h. After treatment, cells were stained with JC-1, a fluorescent probe of MMP, and then the stained H196 cells were analyzed by fluorescence microscopy. The change of MMP was evaluated by the shift of red florescence (JC-1 aggregates) to green florescence (JC-1 Monomer). **b** Auranofin and cisplatin co-treatment obviously induced DNA damage. H69 and H196 cells were treated with DMSO control, auranofin (1 μM), cisplatin (1 μM), or the combination of auranofin and cisplatin for 12 h, and then γH2AX foci formation was detected by immunofluorescence

H69 SCLC were treated by intraperitoneal injection with vehicle control, auranofin (10 mg/kg), cisplatin (2 mg/kg), or the combination of auranofin and cisplatin every two days for 28 days. As shown in Fig. 5a and b, the combination treatment with auranofin and cisplatin significantly inhibited tumor volume of H69 xenografts as compared with vehicle treatment. However, auranofin or cisplatin single treatment did not significantly inhibit tumor growth. Tumor weight of the

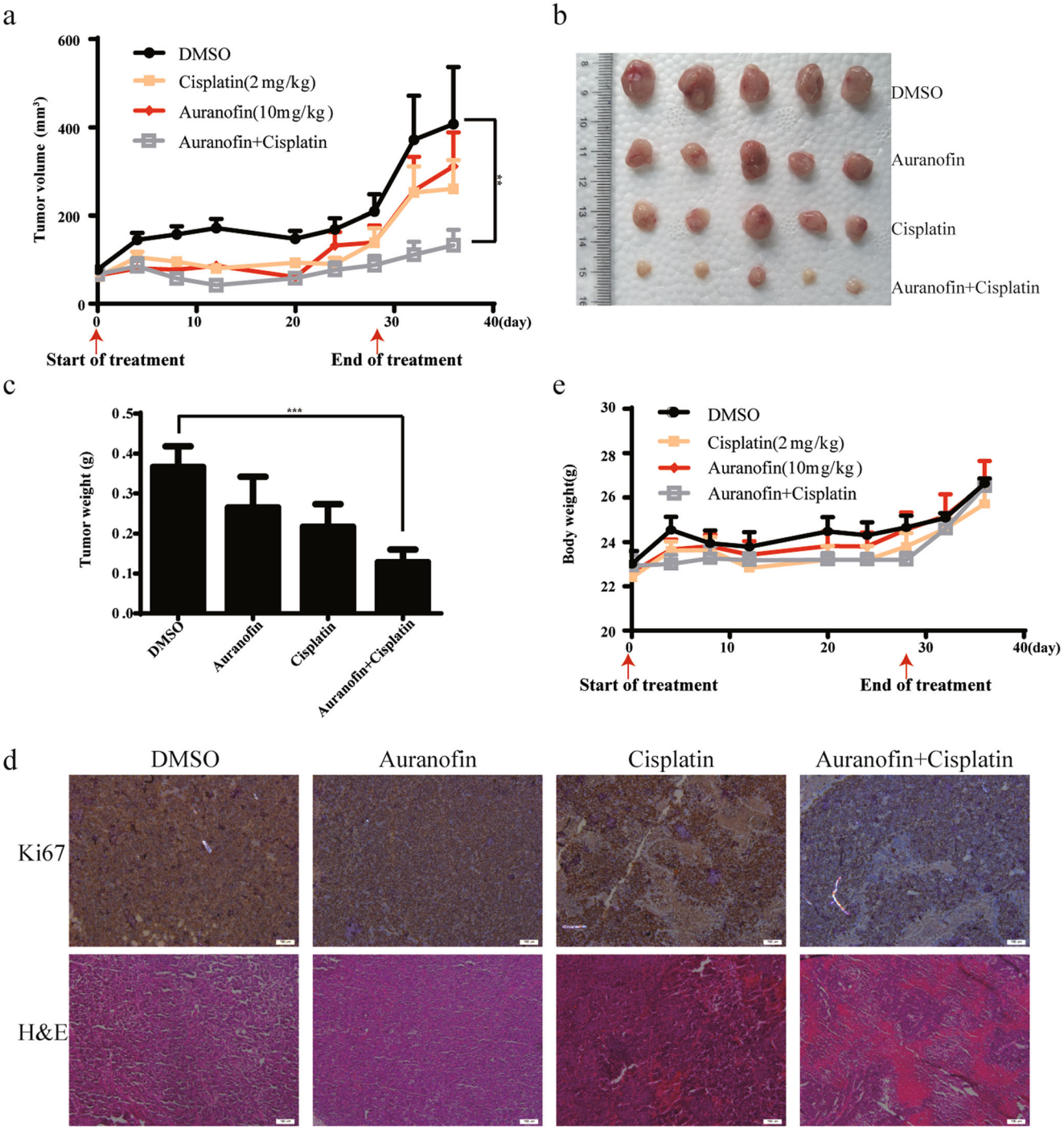



**Fig. 5 Auranofin/cisplatin combination potently reduces SCLC tumor growth in vivo a** The combination treatment of auranofin and cisplatin remarkably inhibited tumor growth in mouse model with SCLC H69 xenografts. Mice with H69 xenografts were treated by DMSO control, 10 mg/kg auranofin, 2 mg/kg cisplatin, or the combination of auranofin and cisplatin every two days for 4 weeks. The mean tumor size ± SEM is shown. (**, $P < 0.01$ by an unpaired t test). **b** Imaging of tumors from each group. The tumors were excised at the end of the experiment. **c** At the end of the experiment, tumor weight in auranofin/cisplatin co-treatment group was significantly decreased compared to control group. The mean tumor weight ± SEM is shown. (***, $p < 0.001$, by an unpaired t test). **d** H&E staining and immunohistochemistry detection of Ki67. **e** Body weights of the nude mice during treatment

combination-treated mice group was significantly reduced as compared with the vehicle-treated mice group (Fig. 5c). Proliferation marker Ki67 was found to be apparently decreased in the combination group (Fig. 5d). No significant difference in body weight was observed in different treatment groups (Fig. 5e). The in vivo study further demonstrates that the combination of auranofin and cisplatin potently inhibits SCLC tumor growth.

## Discussion

Chemotherapy-resistance is a major challenge for SCLC therapy in clinic. Thus, it is necessary to develop effective therapeutic strategies to overcome SCLC chemo-resistance. In this study, we found that auranofin was capable of enhancing chemosensitivity in SCLC in vitro and in vivo. Furthermore, our study demonstrated that auranofin sensitized SCLC to cisplatin through ROS overproduction, as a classic ROS scavenger NAC abolished chemo-sensitization of auranofin in SCLC.

Auranofin is a clinical-approved drug that is used for the treatment of rheumatoid arthritis [14]. Previous study has found that auranofin was able to inhibit thioredoxin reductase (TrxR), thereby inducing the production of ROS [15, 16]. Comparing with normal cells, cancer cells usually have a higher level of ROS and antioxidant activity in a balance status. Cancer cells are unable to tackle additional oxidative stress and become vulnerable to ROS [17, 18]. A recent study has indicated that breast cancer stem cells were sensitive to glycolysis inhibitor 2-DG, a ROS inducer, when thioredoxin (TXN) and glutathione (GSH) antioxidant pathways were inhibited [19]. Yan et al. have reported that lung cancer with the deficiency of glutathione (GSH) antioxidant pathways was more sensitive to auranofin [20]. Hatem et al. have reported that auranofin both inhibited thioredoxin and glutathione antioxidant systems, and the combination of auranofin and Vitamin C (a ROS generator) exerted a synergistic antitumor activity in triple-negative breast cancer [21]. Therefore, targeting antioxidant pathway is an important strategy for cancer therapy.

Our study demonstrated that auranofin synergized cisplatin through ROS overproduction, which lead to mitochondrial dysfunction and DNA damage in SCLC. Wang et al. have reported that auranofin radiosensitized tumor cells through ROS overproduction [13]. In gastric cancer, Zou et al. have demonstrated that auranofin induced apoptosis by increasing intracellular ROS level, which further lead to mitochondrial dysfunction [12]. ROS overproduction also induced DNA damage. Excessive generation of ROS enhanced DNA damage caused by chemo-drugs in breast cancer cells, chronic lymphocytic leukemia and lung adenocarcinoma cells [22–24]. Therefore, our study suggests that auranofin can enhance the sensitivity of chemotherapy through ROS overproduction.

Currently, auranofin has been clinically tested to be combined with mTOR inhibitor sirolimus in treating patients with advanced or recurrent NSCLC and SCLC (ClinicalTrials.gov Identifier: NCT01737502). Our study warrants further clinical study to test whether auranofin sensitizes SCLC to cisplatin, a routine chemodrug.

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## Compliance with ethical standards

**Conflict of interest** Authors have no financial/commercial conflicts of interest regarding the study.

**Ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

This article does not contain any studies with human participants performed by any of the authors.



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