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Oridonin inhibits oral cancer growth and PI3K/Akt signaling pathway

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ABSTRACT

Oridonin, a bioactive diterpenoid purified from *Rabdosia rubescens*, has been shown to possess anticancer capacity in several cancer types. However, its effects on oral squamous cell carcinoma (OSCC) cells remain unclear. This study aimed to investigate the anticancer ability of oridonin in OSCC cells, including proliferation, apoptosis and underlying mechanisms using the OSCC cell lines, UM1 and SCC25. The results showed that oridonin not only inhibited proliferation and clonal formation but also induced G2/M cell cycle arrest and apoptosis in UM1 and SCC25 cells in a dose-dependent manner. Western blot revealed that oridonin treatment increased the ratio of Bax/Bcl-2, and activated the cleavage of caspase-3, caspase-9 and PARP-1. Oridonin also induced G2/M phase arrest in OSCC cells *via* down-regulating the G2/M transition-related proteins such as cyclin B1 or up-regulating cyclin D1, cyclin D3, P21, p-CDK1 and cyclin A2. In addition, oridonin treatment significantly inhibited the phosphorylation of PI3K and Akt and inhibited tumor growth of OSCC xenograft in nude mice. Taken together, these results suggested that oridonin possesses anti-oral cancer capacity *via* inhibiting the PI3K/Akt signaling and induce apoptosis and G2/M-phase arrest. Therefore, oridonin may be a potential anticancer drug for the treatment of oral cancer.

1. Introduction

Oral cancer is the sixth most common cancer worldwide with a high mortality rate. According to the World Health Organization (WHO), it is estimated that approximately more than 300,000 patients are newly diagnosed with oral cancers worldwide each year, accounting for 2–3% of all malignancies [1]. Oral squamous cell carcinoma (OSCC) is the most common type of oral cancer, accounting for 96% of all cases [2]. Most OSCC cases are diagnosed at an advanced stage (III and IV), which 5-year survival rate is less than 50% [3]. Even more, for the untreated and metastatic OSCC, the survival time is only about four months [3]. Therefore, early detection and treatment procedure are crucial for improving the survival of patients [4].

Current treatment options for OSCC include surgical treatment, radiotherapy and combined chemotherapy such as cisplatin, carboplatin, 5-uracil, paclitaxel [5]. However, chemotherapy is easy to induce drug resistance, thus affecting the patient's compliance and therapeutic efficacy [6,7]. Although recent studies have made some progress in the treatment of OSCC, the prognosis of patients remains not significantly improved [8]. Therefore, it is urgently required to identify more effective chemotherapy drugs.

Oridonin, an *ent*-kaurane tetracyclic diterpenoid, is isolated from the leaves of *Rabdosia rubescens* [9], having a variety of pharmacological and physiological activities, such as anti-inflammatory, anti-bacterial and anti-tumor effects [10,11]. The anticancer ability of oridonin has been demonstrated in a variety of cancer cells, including gastric cancer, colorectal carcinoma, breast cancer, ovarian cancer, pancreatic cancer, non-small cell lung cancers, acute leukemia, glioblastoma multiforme and human melanoma cells [12–18]. It is known that the α-methylene cyclopentanone is the active center of the anticancer activity of oridonin [19]. Oridonin can also induce cell cycle arrest and apoptosis in oral cancer cells [20]. However, the exact effects and the underlying mechanisms of oridonin on human OSCC remains poorly characterized. In the present study, therefore, we aimed to explore the anticancer effects of oridonin and its underlying molecular mechanism in human OSCC cells.

2. Materials and methods

2.1. Cell culture

The human OSCC cell lines, SCC-25 cells (tongue squamous carcinoma cells), UM1, UM2 (oral carcinoma cells), HSC-3 (human oral squamous carcinoma cells), and Cal 27 (tongue squamous carcinoma cells) were obtained from ATCC (Manassas, VA, USA). HSC-3 and Cal 27 cells were cultured in Dulbecco's modified Eagle's (DMEM) medium

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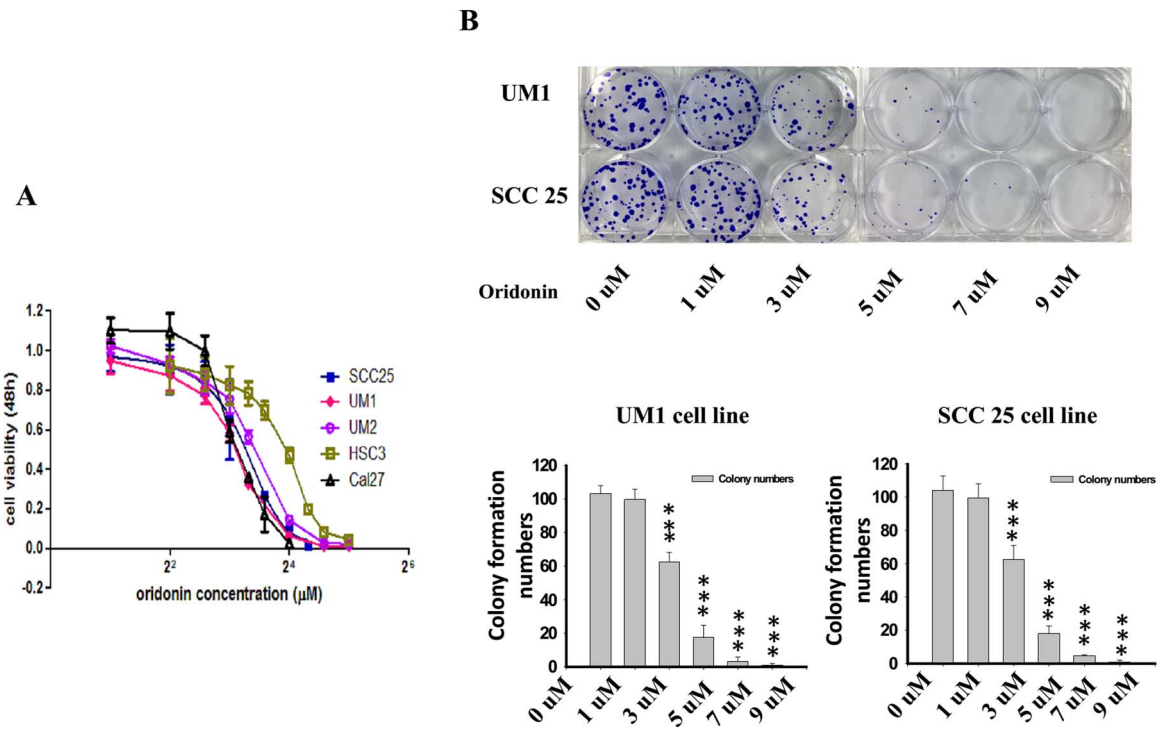

Fig. 1. Oridonin inhibited proliferation and colony formation of oral cancer cells. (A) SCC-25, UM1, UM2, HSC-3 and CAL27 (5×10^3 cells/ well) were seeded onto 96-well-plates. After 24 h, cells were treated with oridonin 1, 3, 5, 7 and 9 μM for 48 h. Cell viability was determined using CCK8 assay. (B) OSCC cells were seeded onto 6-well plates at 500 cells/well and treated with or without oridonin (1, 3, 5, 7 and 9 μM). The colonies were stained with 0.2% crystal violet for 15 min. The bar chart showed the quantitative result. ***P < .001, compared to the control group.

(Gibco, Grand Island, NY, USA). UM1 and UM2 cell lines were cultured in DMEM/F12 (Gibco, USA). SCC-25 cell line was cultured in DMEM/F12 with 0.2% hydrocortisone (Sigma-Aldrich, Louis, MO, USA). All culture mediums contained with 10% fetal bovine serum and 1% penicillin/streptomycin and maintained at 37 °C in a humidified atmosphere of 5% CO_2.

2.2. Cell viability assay

Oridonin (purity > 98%) was purchased from Chengdu Herbpurify CO (Chengdu, China) and dissolved in dimethyl sulfoxide (DMSO) at a stock concentration of 4 mM. Cells were seeded in 96-well plate at a density of 5×10^3 cells/well overnight and then replaced with the fresh medium and/or oridonin 1, 3, 5, 7 and 9 μM for 48 h, respectively. After which, the experiment substrates of Cell Counting Kit-8 (CCK8, EnoGene, NanJing, China) were added 10 μl/well for 1–4 h at 37 °C. The spectrometric absorbance at 450 nm was measured using an automatic enzyme analyzer (Thermo Electron, USA).

2.3. Colony formation assay

Cells were seeded in 6-well plates with a density of 500 cells/well. After 24 h, the culture medium was replaced with fresh medium containing vehicle (control) or different concentrations of oridonin. The medium was changed to complete medium every 2 days. When colony formations were observed in the well-plate, the supernatants were discarded and cell clones were fixed with 4% paraformaldehyde (Guangzhou Chemical Reagent Factory, China) for 10 min. After washing 2 times with PBS, cell clones were stained with 0.2% crystal violet for 30 min, followed by washing with tap water for three times. More than 50 cells of colonies were counted under a microscope (Zeiss, Germany).

2.4. Western blot

The OSCC cells treated with different concentrations of oridonin (90, 9, 18 μM) for different time points were collected for lysis on ice in RIPA buffer. After centrifugation at 12,000 × *g* for 15 min, the supernatant was collected and the protein concentration was determined by BCA Protein Assay Kit (Beyotime, China). Equal amount of total protein was separated on an SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride (PVDF) membrane (Bio-Rad, Hercules, CA, USA). The membrane was blocked with blocking buffer (5% non-fat milk powder in Tris-buffered saline/Tween 20) for 1 h at room temperature and then incubated with primary antibodies against P21 (1:1000, Cell Signaling Technology, USA), GAPDH (1:1000, Cell Signaling Technology, USA), cyclin B1 (1:1000, Cell Signaling Technology, USA), cyclinA2 (1:1000, Cell Signaling Technology, USA), cyclin D1 (1:1000, Cell Signaling Technology, USA), p-CDK1 (1:1000, Cell Signaling Technology, USA), Bax (1:1000, Cell Signaling Technology, USA), BCL-2 (1:1000, Cell Signaling Technology, USA), caspase 3 (1:1000, Cell Signaling, USA), Cleaved Caspase3 (1:1000, Cell Signaling, USA), Cleaved Caspase 9 (1:1000, Cell Signaling, USA), PARP (1:1000, Cell Signaling, USA), Cleaved PARP (1:1000, Cell Signaling, USA), p-PI3K (1:1000, Cell Signaling Technology, USA), Akt (1:1000, Cell Signaling Technology, USA), p-Akt (1:1000, Cell Signaling Technology, USA) at 4 °C overnight. Secondary antibodies were detected with goat anti-rabbit/mouse IgG (CST) antibodies (1:5000, Cell Signaling Technology, USA) conjugated to horseradish peroxide. Immunoblots were detected using ECL. Densitometric analysis for the quantification of the bands was performed using Quantity One software (Bio-Rad Laboratories, USA).

2.5. Flow cytometry

To analyze the cell cycle and apoptosis of the OSCC cells, flow cytometry was carried out. For cell cycle analysis, cells were centrifuged, counted for 2×10^5–1×10^6, and washed the cell pellets with PBS. The