Abstract. Previous studies have demonstrated that oridonin, a tetracyclic diterpenoid compound extracted from *Rabdosia rubescens*, inhibits proliferation and induces apoptosis in several tumor cell lines. However, the mechanism by which oridonin inhibits the cell cycle remains poorly understood. In the present study, possible mechanisms by which oridonin affects cell cycle progression were explored in A549 lung cancer cells. Flow cytometry analysis indicated that oridonin inhibited the proliferation of A549 cells by inducing G2/M cell cycle arrest in a dose-dependent manner. Western blot analysis revealed that in oridonin treated cells, phosphorylated (p-)ATM serine/threonine kinase (S1981), p-checkpoint kinase 2 (CHK2) (T68), p-p53, and phosphorylated H2A histone family member X protein levels were visibly increased, indicating that oridonin promoted G2/M arrest in A549 cells through the ATM-p53-CHK2 pathway. This data suggests that oridonin promotes G2/M arrest in A549 cells by facilitating ATM activation, which is likely a common mechanism in other tumor cell types when using this drug for cancer treatment.

Introduction

Lung cancer is a common malignancy and the leading cause of cancer-associated mortality worldwide (1). Non-small cell lung cancer (NSCLC) accounts for ~80% of lung cancers (2). Despite tremendous progress being made in surgical techniques, chemotherapeutic agents, radiotherapy and novel molecular targeted drugs in previous decades, the prognosis of NSCLC remains poor, making the development of comprehensive treatments for the disease an urgent requirement. Oridonin, alternately known as guidongnin, is a tetracyclic diterpenoid compound extracted and purified from a traditional Chinese herb, *Rabdosia rubescens*, which is a member of the *Salvia* family. Previous studies have demonstrated that oridonin inhibits the proliferation of A549 cells by inducing G2/M cell cycle arrest in a dose-dependent manner. Western blot analysis revealed that in oridonin treated cells, phosphorylated (p-)ATM serine/threonine kinase (S1981), p-checkpoint kinase 2 (CHK2) (T68), p-p53, and phosphorylated H2A histone family member X protein levels were visibly increased, indicating that oridonin promoted G2/M arrest in A549 cells through the ATM-p53-CHK2 pathway. This data suggests that oridonin promotes G2/M arrest in A549 cells by facilitating ATM activation, which is likely a common mechanism in other tumor cell types when using this drug for cancer treatment.

Materials and methods

Reagents and antibodies. Oridonin (≥98%) was purchased from Shanghai Standard Technology Co., Ltd. (Shanghai, China) and dissolved in dimethyl sulfoxide (DMSO; final concentration ≤0.1%). Dulbecco's modified Eagle's medium (DMEM) and calf serum were obtained from Gibco; Thermo

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Oridonin induces cell cycle arrest at G2/M phase in A549 cells. To determine whether oridonin-induced cell cycle arrest contributes to tumor growth inhibition, A549 cells were exposed to oridonin at different concentrations (16, 32 and 64 µmol/l) for 48 h, and same volume of DMSO was administered as a vehicle control. Cells were subsequently harvested and cell cycle activity assessed by flow cytometry (Fig. 1). Quantitative analysis revealed that the proportion of G2/M phase cells was significantly increased, dose-dependently, in oridonin-treated A549 cells treated with 16 (P=0.014; Table I), 32 (P=0.009; Table I) and 64 µmol/l oridonin (P=0.00003; Table I) compared with DMSO-treated cells. By contrast, the proportion of G0/G1 phase cells decreased as a result of oridonin treatment compared with DMSO treatment (Table I). These results demonstrated that oridonin arrests A549 cells at the G2/M phase of the cell cycle in a dose-dependent manner.

Oridonin induces G2/M arrest by facilitating ATM activation in A549 cells. To investigate whether cell cycle-associated proteins (γ-H2AX, H2AX, lamin-B, p-ATM, p-CHK2, p-CHK2, p-p53 and p53) are involved in the oridonin-induced G2/M arrest in A549 cells, western blot was performed to evaluate the level of each protein. Following treatment with 16, 32 and 64 µmol/l oridonin, p-ATM (S1981); p-CHK2 (S68), CHK2, p-p53 and p53) are involved in the oridonin-induced G2/M arrest in A549 cells, western blot was performed to evaluate the level of each protein. Following treatment with 16, 32 and 64 µmol/l oridonin, p-ATM (S1981), p-CHK2 and p-p53 were detected by flow cytometry (Cytoflex, Beckman Coulter, Inc., Brea, CA, USA). RNase and propidium iodide (PI) solution were purchased from Sigma-Aldrich; Merck Millipore (Darmstadt, Germany).
A549 cells increased (Fig. 2). The higher the concentration of oridonin used, the greater upregulation of p-ATM (S1981), p-CHK2 (T68), p-p53, p53 and γ-H2AX protein expression levels was presented (Fig. 2).

Effects of oridonin on p-ATM (S1981) and γ-H2AX. To further explore the function of p-ATM (S1981) and γ-H2AX on cell cycle arrest, A549 cells were treated with 16, 32 and 64 µmol/l oridonin and immunostaining was performed to investigate changes in expression of these two proteins. Both p-ATM (S1981) and γ-H2AX were upregulated following oridonin treatment (Fig. 3). Both p-ATM (S1981) and γ-H2AX were localized in the cell nucleus (Fig. 3).

Discussion

Lung cancer is one of the most common malignant tumors, and is associated with poor overall survival, therefore research into effective treatments is required (16). Previous in vitro studies have explored the anti-tumor effects of oridonin, and have demonstrated that it inhibits abnormal cell proliferation and induces apoptosis in various human tumor cell lines (6,8,9,17-23).

The cell cycle is a fundamental cellular event, which is regulated at multiple levels by various factors in vitro and in vivo. Cell proliferation, division, apoptosis and necrosis
of DNA damage signals to downstream proteins of signal transduction and act on effector proteins, which may result in distinct effects including cell cycle arrest, apoptosis, DNA repair and transcriptional program activation induced through the ATM-p53-CHK2 pathway (36). As mentioned previously, DNA damage induces H2AX phosphorylation, which is recognized as a DNA damage biomarker (37). In the present study, immunofluorescence staining data confirmed that oridonin treatment induced DNA damage, with increased levels of γ-H2AX in the nucleus of treated cells. Increased p-ATM (S1981) in the nucleus following oridonin treatment demonstrated that DNA damage resulted in increased levels of activated ATM in the cell nucleus.

To the best of our knowledge, this is the first report to demonstrate that oridonin-induced A549 cell cycle arrest occurs via activation of the ATM signaling pathway, although there are some limitations present. Downstream effector protein expression levels were not evaluated by western blotting, which prevented the investigation of detailed mechanisms underlying oridonin-induced cell cycle arrest in A549 cells. In addition, it remains unclear if ATM or CHK2 directly induced the activation of p53. Further experiments are required to address these questions.

In conclusion, the present study demonstrated that oridonin induces G2/M cell cycle arrest through activating the ATM signaling pathway to inhibit proliferation in A549 cells. Future investigations may determine whether oridonin treatment induces the apoptosis of A549 cells.

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References


