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Costunolide, an active sesquiterpene lactone, induced apoptosis via ROS-mediated ER stress and JNK pathway in human U2OS cells

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ABSTRACT

Costunolide, an active sesquiterpene lactone, is derived from many herbal medicines and it exhibits a broad spectrum of bioactivities such as anti-inflammatory, potential anti-tumor activity. Herein we assessed the anti-cancer effects of costunolide on U2OS cells and explored the underlying molecular mechanisms. The experiment data show that Costunolide exhibited significant anti-tumor activity by apoptosis related assays including Annexin V-FITC/PI flow cytometric analysis and 4,6-diamino-2-phenyl indole (DAPI) staining morphological analysis. Furthermore, we found Costunolide induced the loss of mitochondrial transmembrane potential, down-regulated Bcl-2/Bax ratio, encouraged Cyt-c release and caspase activation. All those effects are contributed by reactive oxygen species (ROS) generation and ER stress-induced mitochondrial dysfunction which are also responsible for c-Jun N-terminal kinase (JNK) activation. After the treatment of JNK inhibitor SP600125, it obviously reversed costunolide-induced apoptosis. Given *N*-acetyl-L-cysteine (NAC) effectively blocked the activation of JNK. Taken together, our results demonstrate that costunolide induces apoptosis in human U2OS cells through ROS generation and p38 MAPK/JNK activation.

1. Introduction

Osteosarcoma (OS) is regarded as one of the most common malignant solid bone cancers, occurring predominantly in children and young adults, with a peak incidence in the second decade of life [1]. Osteosarcoma is unstable and heterogenous because of its high rate of metastasis, nearly 25% to 50% for patients with lung or bone metastases [2,3]. However, the prognosis for patients with metastatis is poor and unwanted side effects of chemotherapy may result in dose reduction or treatment interruption [4]. To develop more effective drugs against OS is imperative and necessary.

Herbal medicines have long been a crucial source for anti-cancer agents, and some are currently used in clinical practice [5]. Costunolide, derived from many herbal medicines, exhibiting a broad spectrum of bio activities such as anti-inflammatory, anti-oxidant properties and anti-tumor activity [6,7]. Published reports have described that costunolide exerted anti-oxidant effects by inhibiting the expression of inducible nitric oxide synthase and the DNA-binding activity of NF-κB [8]. Compared with normal cells, cancer cells can be selectively killed because of its higher levels of endogenous Reactive oxygen species (ROS) and the accessible toxic threshold [9–11]. Therefore, intracellular ROS burst triggers apoptosis. Previous studies demonstrated that costunolide could increase ROS generation in breast cancer cells [12], human bladder cancer cells [13], ovarian cancer cells [14] and so on. It is generally believed that there are two major apoptotic pathways in OS including death-receptor pathway and mitochondrial pathway. Meanwhile, the latter pathway is triggered by the increasing cytochrome C (Cyt-c) in cytoplasm and Bcl-2 gen family, which includes pro-apoptosis (Bax) and anti-apoptosis (Bcl-2) members [15,16]. Recently, numerous reports claimed that increased ROS also induced endoplasmic reticulum (ER) stress in apoptotic execution [17]. On account of up-regulation of BH3 only proteins, blocks ER-mitochondrial contact sites, leading to mitochondrial dysfunction [18]. Several studies have demonstrated that apoptotic cell death induced by ROS is mediated by p38 Mitogen Activated Protein Kinase (MAPK) and JNK activation [19–21]. Three major MAPK subfamilies have been described: p38, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK). The MAPK signaling plays a critical role in the

Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole; OS, osteosarcoma; ROS, reactive oxygen species; ER, endoplasmic reticulum; MAPK, mitogen activated protein kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; ASK1, apoptosis signal-regulating kinase 1; DMSO, dimethyl sulfoxide; ΔYm, Mitochondrial transmembrane potential; MMP, Mitochondrial membrane potential.

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outcome and the sensitivity to anticancer therapies. Activated MAPK can transmit extracellular signals to regulate cell growth, proliferation, differentiation, migration, apoptosis and so on. Apoptosis is one of the major outcomes of MAPK activation exposed with stressors by promoting the transcription of Bcl-2 family proteins, such as Bax and Bcl-2 [22,23]. Apoptosis signal-regulating kinase 1 (ASK1), an upstream kinase of p38 MAPK, is thought to be essential for ROS-mediated apoptosis in a broad range of cells, also activated in the cells treated with anti-tumor compounds [24,25].

In this study, we assessed anti-cancer effects of Costunolide. Our data indicated that Costunolide elevated the level of apoptosis in U2OS cells through ER-mitochondrial pathway via ROS activated the MAPKs.

2. Materials and methods

2.1. Cell culture and reagents

Human osteosarcoma U2OS cells, human alveolar adenocarcinoma A549 cells and Hela cells were purchased from American Type Culture Collection (ATCC, USA). U2OS and A549 cells were cultured in DMEM medium (Invitrogen, Carlsbad, CA, USA), while Hela cells were maintained in RPMI-1640 medium (Gibco, USA), containing 10% heat-inactivated fetal bovine serum (Gibco, USA) in a humidified atmosphere of 5% CO_2 at 37 °C.

Antibodies directly against β-actin, JNK, *p*-JNK, Bcl-2 and Bax were from Bioworld (USA). Antibodies of Bip, IREα, *p*-ASK1, *p*-Bcl-2, P38, *p*-P38, ERK, *p*-ERK, Cleaved-PARP and Cyt-c were obtained from Cell Signaling Technology (Beverly, MA, USA). Costunolide (Abcam, USA) was dissolved in DMSO (dimethyl sulfoxide, Sigma-Aldrich, St. Louis, MO, USA) to make a stock solution. MTT (Sigma, 88417) was dissolved in 10 mM phosphate-buffered saline (PBS) to a concentration of 5 mg/mL. 4,6-diamino-2-phenyl indole (DAPI) was purchased from Santa Cruz (Santa Cruz, CA). NAC (*N*-acetyl-L-cysteine) purchased from Sigma Aldrich (USA) was dissolved in sterile water.

2.2. Cell morphological assessment

The U2OS cells were plated in 6-well incubated overnight. To detect morphological features of apoptosis, cell nuclei were

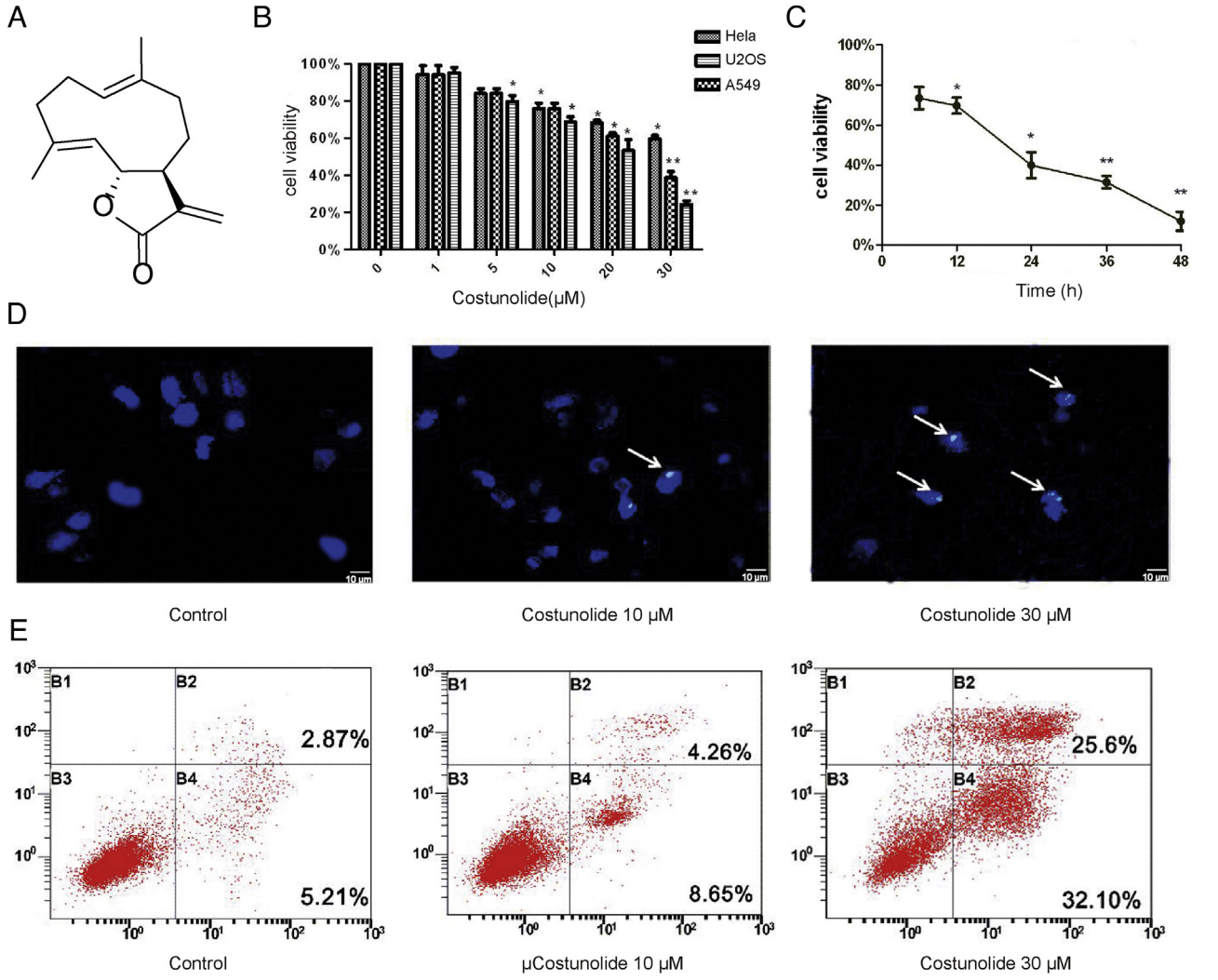

Fig. 1. Costunolide induced apoptosis in human osteosarcoma U2OS cells. (A) Structure of Costunolide ($C_{15}H_{20}O_2$). (B) MTT assay of Hela, A549 and U2OS cells. (C) MTT assay of U2OS cells at specific times after given Costunolide 30 μM. (D) The nucleolus morphologic changes were observed by fluorescent microscope in U2OS cells (×400). (E) The apoptotic rates of cells induced by Costunolide in U2OS cells detected by Annexin V/PI double-staining assay. Data were shown as means ± SD for three independent experiments (*$p < 0.05$ and **$p < 0.01$ compared with control).