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Cucurbitacin B suppresses metastasis mediated by reactive oxygen species (ROS) *via* focal adhesion kinase (FAK) in breast cancer MDA-MB-231 cells

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[ABSTRACT] Metastasis is responsible for the majority of cancer-related deaths and prevention of metastasis remains a big challenge for cancer therapy. Cucurbitacin B (Cuc B) is a natural triterpenoid with potent anticancer activities while its effect on metastasis remains unclear. In the present study, the inhibitory effect and mechanisms of Cuc B on metastasis were investigated in MDA-MB-231 breast cancer cells. The cells were treated with or without Cuc B, and the cytotoxicity was determined by MTT assay. The effect of Cuc B on metastasis was evaluated with wound healing, transwell, and adhesion assays. Furthermore, the adhesion of cancer cells to endothelial cells was determined. The protein expression was determined by Western blotting. Cuc B (< 100 nmol·L^{-1}) showed no obvious cytotoxicity to MDA-MB-231 cells, but significantly inhibited migration, invasion, and adhesion to Matrigel, fibronectin, type I collagen, and endothelial cells. Cuc B dramatically inhibited the phosphorylation of focal adhesion kinase (FAK) and paxillin in dose- and time-dependent manners. Furthermore, Cuc B induced intracellular reactive oxygen species (ROS) generation, which could be reduced by *N*-acetyl-l-cysteine (NAC). In addition, NAC pretreatment could reverse Cuc B-induced suppression of migration and adhesion, expression of FAK, but showed no effect on paxillin expression. In summary, Cuc B suppressed ROS-dependent metastasis through FAK pathway in breast cancer MDA-MB-231 cells, demonstrating novel mechanisms for the anticancer effects of Cuc B.

[KEY WORDS] Cucurbitacin B; Metastasis; ROS; FAK; Breast Cancer

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Introduction

Breast cancer is the most common cancer in women with an incidence rate of over 1.6 million cases per year worldwide [1]. With the development of medical technologies, breast cancer has become one of the most curative cancers with significant improvement extending patient life. However, breast cancer cells could easily migrate to other tissues and organs such as brain [2], bone [3], and liver [4], causing that 30%−40% of patients may eventually suffer from distant relapse and succumb to the disease [5]. Thus, understanding of metastasis biology and suppressing of metastasis have become one of the most challenging areas for the treatment of breast cancer.

Cucurbitacin B (Cuc B, Fig. 1A), the most abundant member of the cucurbitacin family, is a tetracyclic triterpenoid widely distributed in the plant kingdom. A large number of studies have demonstrated that Cuc B and its analogues exert various pharmacological effects on inflammation, hepatoprotection, and especially on cancer [6-7]. Previous studies have reported the anticancer effects of Cuc B on various human cancer cells, including hepatoma, osteosarcoma, laryngeal squamous carcinoma, lung cancer, colon adenocarcinoma, neuroblastoma and leukemia [8-14]. Nearly all these anticancer functions of Cuc B were accomplished by inhibiting the cancer cell proliferation and inducing cell death through apoptosis, autophagy, cell cycle arrest, DNA damage, reactive oxygen species (ROS) formation, and interfering F-actin [15-20].

Previous studies have shown that Cuc B inhibits cell mi

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These authors have no conflict of interest to declare.

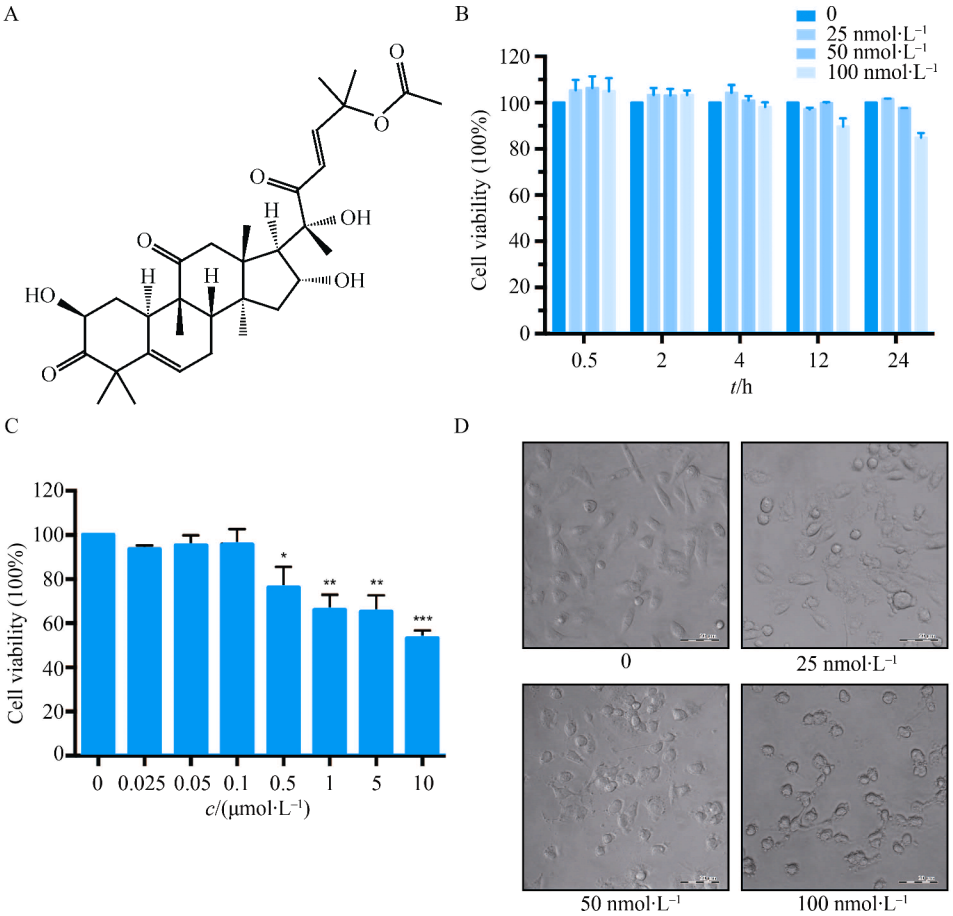

Fig. 1 The cytotoxic effects of Cuc B. The chemical structure of Cuc B (A) and the effect of Cuc B on MDA-MB-231 cell viability (B and C) and morphology (D). Cells were treated with Cuc B (0−100 nmol·L^{-1}) and the cell viabilities were determined by MTT assay. Cells were treated with Cuc B (0−10 nmol·L^{-1}·) for 24 h and the cell viabilities were determined by MTT assay. The cells were treated with Cuc B (0−100 nmol· L^{-1}) for 2 h and the cell morphology was observed with a microscopy. Cuc B, Cucurbitacin B; Con, concentration. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ *vs* control group

gration in cutaneous squamous cell carcinoma lines [21]. In human hepatoma cell lines HepG2 and BEL-7402, Cuc B inhibits 12-*O*-tetradecanoylphorbol 13-acetate-induced invasion and migration through inactivating mitogen-activated protein kinase and PI3K/Akt signal transduction pathways [22]. More recently, it has been shown that Cuc B exerts strong anti-migratory and anti-invasive abilities against metastatic non-small cell lung cancer by downregulation of canonical Wnt/*β*-catenin signaling axis [23] and inhibits breast cancer metastasis and angiogenesis through VEGF-mediated suppression of focal adhesion kinase/matrix metallopeptidase-9 (FAK/MMP-9) signaling axis [24]. Our previous studies and others have demonstrated the important roles of Cuc B-induced reactive oxygen species (ROS) in its anticancer activities [10, 13, 18, 25-26]. We therefore hypothesized that ROS might contribute to its anti-metastatic effects. In the present study, the effects of Cuc B on metastasis in MDA-MB-231 breast cancer cells were investigated and the potential mechanisms were explored.

Materials and Methods

Test compounds, reagents, and antibodies

Cuc B (> 98%) was purchased from Chenguang Herbpurify CO., Ltd. (Chengdu, China). Dulbecco's Modified Eagle Medium (DMEM), Vascular Cell Basal Medium, Endothelial Cell Growth Kit-BBE, fetal bovine serum (FBS), Penicillin-Streptomycin solution (PS), and 5-(6)-carboxy-2′, 7′-dichlorodihydrofluorescein diacetate ($DCFH_2$-DA) were purchased from Sigma (St. Louis, MO, USA). *N*-acetyl-L-cysteine (NAC) was purchased from Beyotime (Haimen, China). Specific antibodies against phosphorylated FAK (Tyr397) (p-FAK), FAK, phosphorylated Paxillin (Tyr118) (p-Paxillin), Paxillin, and GAPDH were purchased from Cell Signaling Technology (Danvers, MA, USA). Matrigel, fibronectin, and type I collagen were purchased from BD Biosciences (San Jose, CA, USA).

Cell culture

The MDA-MB-231 cells originated from American Type Culture Collection (ATCC, USA) were cultured in DMEM supplemented with 10% FBS and 1% PS, growing in an in-