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Effects of triptolide from *Tripterygium wilfordii* on ERα and p53 expression in two human breast cancer cell lines

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Abstract

The aim of the study was to discover possible differential cytotoxicity of triptolide towards estrogen-sensitive MCF-7 versus estrogen-insensitive MDA-MB-231 human breast cancer cells. Considering that MCF-7 cells express functional Estrogen receptor α (ERα) and wild-type p53, whereas MDA-MB-231 cells which are ERα-negative express mutant p53, the anti-proliferation effect of triptolide on MCF-7 and MDA-MB-231 cells were examined, the apoptotic effect and cell cycle arrest caused by triptolide were investigated, ERα and p53 expression were also observed in this paper. The results showed that the anti-proliferation effects were induced by triptolide in both cell lines. But the value of IC_{50} in MCF-7 cells for its anti-proliferation effect was about one tenth of that in MDA-MB-231 cells, which indicated that the effect is more potent in MCF-7 cells. Condensed chromatin or fragmented nuclei could be found in MCF-7 cells treated with only 40 nM triptolide but in MDA-MB-231 cells they couldn't be observed until the concentration reached to 400 nM. Triptolide induced significant S cell cycle arrest along with the presence of sub-G0/G1 peak in MDA-MB-231 cells, whereas there was only slightly S cell cycle arrest on cell cycle distribution in MCF-7 cells. The role of p53 in two breast cancer cells was examined, the results showed that the mutant p53 in MDA-MB-231 cells was suppressed and the wild-type p53 in MCF-7 was increased. Moreover, triptolide could down regulate the expression of ERα in MCF-7 cells. The results showed that triptolide is much more sensitive to ERα-positive MCF-7 cells than to ERα-negative MDA-MB-231 cells, and the sensitivity is significantly associated with the ERα and p53 status.

Keywords: Triptolide; *Tripterygium wilfordii*; Estrogen receptor alpha; P53; Human breast cancer cells

Introduction

Triptolide, a tri-epoxide phenanthrene (Fig. 1) and a major component in Chinese herb *Tripterygium wilfordii* Hook F (TWHF), has multiple biological effects. In addition to its potent immunosuppressive and anti-inflammatory activity (Qiu and Kao 2003; Liu et al. 2006), its antineoplastic activity has been a hot topic in recent years. Triptolide induces apoptosis and inhibits the growth and metastasis in many tumors such as prostate cancer, cholangiocarcinoma and multiple myeloma (Yang et al. 2003; Panichakul et al. 2006; Lou et al. 2005; Tong et al. 2007; Zhang et al. 2007). It has been reported that the antitumor effects of triptolide resulted from the decreased expression of cell-cycle promoting

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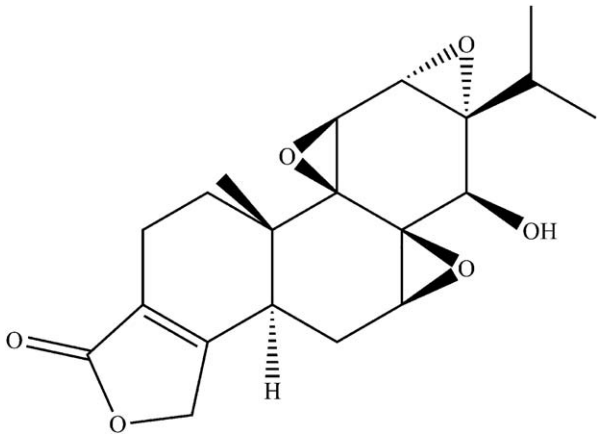

Fig. 1. The structure of triptolide.

factors such as cyclins (A, B1, and D1) and c-myc in breast tumor MDA-435 cells (Miyata et al. 2005). It has been also reported that triptolide can induce cell arrest in the S phase in human fibrosarcoma HT-1080 cells (Fidler et al. 2003) and prostatic adenocarcinoma cells (Kiviharju et al. 2002). In addition, the antitumor effects induced by triptolide has been reported to be associated with the down regulation of NF-KappaB in human multiple myeloma cells (Lou et al. 2005) and with the involvement of mitochondrial pathway in cytotoxicity induced by triptolide in human normal liver L-02 cells (Yao et al. 2008). Furthermore, the *in vitro* and *in vivo* antitumor proliferation caused by triptolide may be associated with apoptosis (Jiang et al. 2001; Chang et al. 2001), but the exact mechanism responsible for the antineoplastic effect of triptolide is not clearly illuminated.

Breast cancer is the main cause of cancer deaths among women (Greenlee et al. 2000). Major advances in breast cancer therapy showed that a number of resistance mechanisms were presented to reduce the effectiveness of chemotherapeutic drugs (Clarke et al. 2005; Evan and Vousden 2001). Therefore, some novel and more effective strategies to prevent and treat breast cancer are still required to be researched. A reasonable treatment plan should be chosen according to the clinical stages, pathological types, tumor positions and the level of estrogen receptors (Wang 1988). The methods of treating the hormone-sensitive breast cancers by inhibiting the estrogen production and its binding to estrogen receptors have been adopted (Jordan and Brodie 2007). Recently, it has been reported that triptolide can inhibit proliferation of MCF-7 human breast cancer cells (Wang et al. 2006). However, the antineoplastic mechanism obtained through comparing the effect of triptolide on different kinds of breast cancers which are greatly impacted by estrogen-sensitivity is not reported yet. It is important to investigate the effect of triploide dependent on ERα and p53 status.

Materials and Methods

Cell culture and triptolide

MDA-MB-231 cells and MCF-7 cells were purchased from Shanghai Institute of Cell Biology and Biochemistry, Chinese Academy of Sciences, China. Cells were grown on tissue culture flasks in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% heat-inactivated fetal bovine serum (Gibco) and antibiotics (100 U/ml penicillin G, 100 ug/ml streptomycin) at 37 °C in 5% CO_2 and 95% air in a humidified incubator. MDA-MB-231 cells and MCF-7 cells designated for experimental usage were grown in culture medium containing serum that had been stripped with Dextran-coated Charcoal (DCC) to remove steroids and other low-molecular weight factors. Triptolide was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (purity >98% (HPLC), Beijing, China).

Cell viability assay

The cells were seeded in 96-well plates at a density of 5×10^4/ml in a humidified incubator with 5% CO_2, 37 °C for 2–3 days. The cells were then exposed to 1-1000 nM triptolide for 24, 48 and 72 h, respectively. At the end of treatment period, 20 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reagent (5 mg/ml in PBS) was added to each well. The plates were then incubated for 4 h at 37 °C in the dark. The medium was removed and MTT reduction product (formazan crystals) was dissolved in Dimethyl Sulfoxide (DMSO), and the absorbance at 490 nm was measured with an ELISA reader (Bio-Rad, USA).

Lactic Dehydrogenase (LDH) release assay

LDH release was determined by measuring changes in absorbance at 530 nm due to NADH oxidation with LDH assay kits (Roche Applied Science). The amount of LDH released into the medium under different treatments compared to the total amount of LDH is defined as cytotoxicity, the total amount of LDH consists of the amount derived from cells treated with 2% Triton X-100 and the amount of LDH released from the medium.

Cell cycle analysis

The distribution of cells at specific cell cycle stages was evaluated by Flow Cytometry. 5×10^5 cells/well were cultured on tissue culture plates with triptolide treatment in a humidified incubator with 5% CO2, 37 °C for 48 h, and cells incubated in medium without