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Rugosin E, an ellagitannin, inhibits MDA-MB-231 human breast cancer cell proliferation and induces apoptosis by inhibiting nuclear factor-κB signaling pathway

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Abstract

In this study, we first report the chemopreventive effect of rugosin E in human breast cancer cell line, MDA-MB-231. Treatment with rugosin E decreased the cell proliferation of MDA-MB-231 cells in a dose-dependent manner. Rugosin E treatment arrested MDA-MB-231 cells at G0/G1 phase. This effect was strongly associated with concomitant decrease in the level of cyclin D1, cyclin D2, cyclin E, cdk2, cdk4, and cdk6, and increase of p21/WAF1. In addition, rugosin E also induced apoptotic cell death. Rugosin E increased in the expression of Bax, Bak, and Bcl-Xs, but decreased the levels of Bcl-2 and Bcl-X_L, and subsequently triggered mitochondria apoptotic pathway (release of cytochrome *c*, activation of caspase-9, and caspase-3). In addition, pre-treatment of cells with caspase-9 inhibitor blocked rugosin E-induced cell proliferation and apoptosis, indicating caspase-9 activation was involved in rugosin E-mediated MDA-MB-231 cells apoptosis. Rugosin E inhibited the constitutively activated and inducible NF-κB in both its DNA-binding activity and transcriptional activity. Furthermore, rugosin E also inhibited the TNF-α-activated NF-κB-dependent reporter gene expression of cyclin D1, c-Myc, XIAP, Bcl-2, and Bcl-X_L were all downregulated by rugosin E. Our results indicated that rugosin E inhibits the activation of NF-κB, and this may provide a molecular basis for drug development in the prevention and treatment of cancer by rugosin E.

Keywords: Rugosin E; Breast cancer; Apoptosis; Cell cycle; NF-κB

1. Introduction

Apoptosis is a multi-step and multi-pathway programmed cell death that is inherent in every cell of the body. In tumors, the ratio of apoptosis to cell

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proliferation is unbalanced, which leads to an increase of malignant tissue. Many studies have demonstrated that cancer treatment by chemotherapy and γ-irradiation kills target cells primarily by the induction of apoptosis [1]. Nuclear factor-κB (NF-κB) is an important transcriptional factor that participates in the regulation of inflammatory, immune, and apoptotic responses [2,3]. A variety of external stimuli induce phosphorylation and subsequent degradation of IκB inhibitory proteins, thereby releasing NF-κB protein for translocation to the nucleus to function as transcription factor [2,3]. NF-κB has been reported to modulate the expression of several genes whose products are associated with tumor development [4,5]. These include anti-apoptotic genes (i.e., c-FLIP, Bfl-1, Bcl-2, Bcl-X_L, and XIAP); cancer invasiveness genes (i.e., matrix metalloproteinases (MMP)-9, urokinase, and COX-2) and cell cycle-related genes (i.e., c-Myc and cyclin D1) [6–8]. NF-κB can also decrease the induction of apoptosis mediated by genotoxic chemotherapeutic agent and ionizing radiation. Cancer cells in which NF-κB is constitutively active are highly resistant to anti-cancer agents or ionizing radiation, and inhibition of NF-κB activity in these cells greatly enhances their sensitivity to anti-cancer treatment [2–4].

Rugosin E (Fig. 1), an ellagitannin, is isolated from *Rosa rugosa* Thunb [9]. Previous studies showed that it was considered as a potent platelet aggregating agent due to its ADP-mimicking effect [10]. In this study, we used a human breast cancer cell line, MDA-MB-231, to evaluate the potential of rugosin E as a chemopreventive agent against breast cancer. Here, we first report on the effects and molecular mechanisms of action of rugosin E in MDA-MB-231 that are mediated through down-regulation of NF-κB signaling pathway.

2. Materials and methods

2.1. Materials

Fetal bovine serum (FBS), penicillin G, streptomycin, amphotericin B, and Dulbecco's modified Eagle's medium (DMEM) were obtained from Gibco-BRL (Gaithersburg, MD). Dimethyl sulfoxide (DMSO), ribonuclease (RNase), and propidium iodide (PI) were purchased from Sigma Chemical (St. Louis, MO). XTT was obtained from Roche Diagnostics GmbH (Mannheim, Germany). Nucleosome ELISA kit, TNF-α, cyclin D1, cyclin D2, cyclin E, cdk2, cdk4, cdk6, Bcl-Xs, Bak, and Bcl-X_L antibody were purchased from Calbiochem (Cambridge, MA). The antibodies to Bcl-2, Bax, XIAP, and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

2.2. Preparation of rugosin E

Rugosin E was isolated from plants according to the procedures described in previous study [9]. Briefly, air-dried bark of *Rosa rugosa* Thunb was chipped into small pieces. A portion of the ethyl acetate extract, which was obtained from the 70% acetone homogenate of *Rosa rugosa*, was chromatographed over Sephadex LH-20 with MeOH-70% aqueous acetone (20:0 → 20:1 → 20:2 → 20:4, v/v), to give rugosin E [9].

Rugosin E was isolated from plants according to the procedures described in previous study [9]. The stock solution of rugosin E was prepared at a concentration of 4000 μM of DMSO. It was then stored at −20 °C until

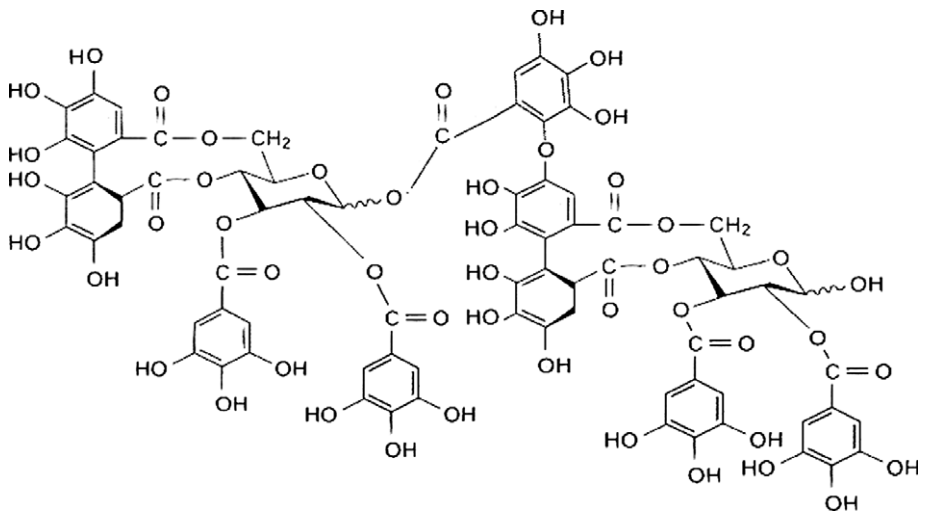

Fig. 1. Chemical structure of rugosin E.