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Decursin from *Angelica gigas* Nakai induces apoptosis in RC-58T/h/SA#4 primary human prostate cancer cells via a mitochondria-related caspase pathway

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ABSTRACT

Decursin is a major biological active component of *Angelica gigas* Nakai and is known to induce apoptosis of metastatic prostatic cancer cells. However, the apoptotic mechanism of decursin using primary malignant tumor (RC-58T/h/SA#4)-derived human prostate cells is not known. In the present study, we show that treatment of prostate cancer cells with decursin inhibited cell proliferation in a dose-dependent manner. Decursin also induced apoptosis in RC-58T/h/SA#4 cells, as determined by flow cytometry, Hoechst 33258 staining, and DNA fragmentation. Decursin caused activation of caspases-8, -9, and -3 and promoted the apoptotic action of caspase-8-mediated Bid cleavage. Decursin increased the protein levels of Bax and cytosolic cytochrome c as well as cleavage of PARP while decreasing the protein levels of Bcl-2. Furthermore, the caspase-independent mitochondrial apoptosis factor, apoptosis-inducing factor (AIF), was upregulated by treatment with decursin. Taken together, these findings indicate that decursin inhibited the proliferation of RC-58T/h/SA#4 cells through induction of apoptosis, which is mediated by both caspase-dependent and -independent apoptotic pathways.

1. Introduction

Prostate cancer is the second leading cause of cancer-related deaths in males in the United States (Adhami et al., 2004). Presently, only some taxane drugs have shown limited efficacy for advanced prostate cancer (Petrylak, 2005). Even so, all of these treatments cause significant side effects. Therefore, non-toxic alternatives are needed to decrease the risk and burden of prostate cancer (Jiang et al., 2006). In the recent past, agents obtained from herbs and plants have gained considerable attention for the prevention and/or treatment of certain cancer types, including prostate cancer (Mukhtar and Ahmad, 1999).

Root of the Korean medicinal herb *Angelica gigas* Nakai is used in traditional oriental herbal medicine for the treatment of gynecological diseases such as menoxenia and anemia (Yamada et al., 1985; Lu et al., 2003). It is also known to have several biological activities, such as anti-amnestic activity (Kang et al., 2003; Kim et al., 2010), inhibitory effect on acetylcholinesterase (Kang et al., 2001), anti-bacterial action (Lee et al., 2003b), anti-allergic effect (Joo et al., 2010), inhibition of VEGF-induced angiogenesis (Jung et al., 2009; Son et al., 2009), and anti-tumor activity against various types of cancer cells (Lee et al., 2003a; Jiang et al., 2007; Ahn et al., 2010) In particular, its anti-tumor activity has recently attracted considerable attention.

A. gigas Nakai contains pyranocoumarin compounds as major active principles, including decursin (Ahn et al., 1997), its isomer decursinol angelate (Konoshima et al., 1968), and decursinol (Jiang et al., 2006). Decursin has been reported to inhibit the growth and survival on LNCaP, DU-145, and PC-3 metastatic prostatic cancer cells (Rhim, 2000; Yim et al., 2005). However, there has been no report on the anticancer activity of decursin against primary prostate cancer cells. Therefore, further studies using primary cells are needed pre-clinical or early clinical research.

In vitro human cell culture models are critical for clarifying the mechanism of prostate cancer progression and for testing preventive and therapeutic agents (Gu et al., 2006).

Readily available and well-studied long-term human prostate cancer cell lines such as PC-3, DU-145, and LNCaP cells derived from bone, brain, and lymph node metastases, respectively (Horoszewicz et al., 1983; Manin et al., 2002; Alimirah et al., 2006). Thus, it is unlikely that these cell lines accurately reflect

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the genetic makeup or biological behavior of primary prostate tumors (Gu et al., 2006). To study early genetic and molecular prostate cancer lesions, cell lines derived from primary tumors are urgently needed.

The RC-58T/hTERT cells were used to develop a novel human cancer culture model for the study of prostate cancer. hTERT-immortalized primary human prostate cancer cell lines maintain the original phenotypes of the primary cells and express some of their prostate-specific markers (Yasunaga et al., 2001). The RC-58T/h/SA#4 cell line was derived from a soft agar clone of the RC-58T/hTERT cell line (Gu et al., 2006).

Therefore, this study aimed to investigate the cytotoxicity of decursin and its potential mechanisms by using primary malignant tumor (RC-58T/h/SA#4)-derived human prostate cells. We found that decursin induced apoptosis via both caspase-dependent and -independent pathways.

2. Materials and methods

2.1. Isolation of decursin from root of Angelica gigas Nakai

Dried roots (100 g) were ground into powder and then extracted three times with 95% methanol by boiling for 3 h at 80 °C. The combined extract (7 g) was suspended between CH_2Cl_2 and H_2O. The organic layer (4.4 g) was subjected to silica gel column chromatography. The column was eluted with solvents of increasing polarity (hexane–ethyl acetate) to obtain six fractions. Fraction 5 was chromatographed again on a silica gel column and the eluted with solvents of increasing polarity (hexane–ethyl acetate) to obtain 35 fractions. The 30 fractions were combined and evaporated to obtain 336 mg of decursin. Decursin with its chemical structure is shown in Fig. 1A.

2.2. Cell culture and cell proliferation

The RC-58T/h/SA#4 (primary prostate cancer cells; androgen positive cells) cells and RWPE-1 (human prostate epithelial cells) were obtained from Center for Prostate Disease Research (CPDR). The cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS), penicillin (100 IU/mL), and streptomycin (100 μg/mL) (Gibco BRL, Life Technologies, Grand Island, NY) in an incubator containing a humidified atmosphere of 5% CO_2 at 37 °C.

Cell proliferation was determined by sulforhodamine B (SRB, Sigma, St. Louis, USA) assay. The cancer cells were seeded at a concentration of 1×10^5 cells/well in 24-well tissue culture plates and incubated with various concentrations of decursin for 48 h. After treatment, medium was aspirated and 10% trichloro-acetic acid was added. After 1 h incubation at 4 °C, the plate was washed five times with D.W and air-dried. The cells were stained with 0.4% (w/v) SRB at room temperature for 1 h and then washed five times using 1% acetic acid. Bound SRB was solubilized with 10 mM Tris, and the absorbance was measured at 540 nm using a microplate reader (Molecular Devices, Inc., USA).

2.3. Detection of morphological apoptosis

Characteristic apoptotic morphological changes were assessed by fluorescent microscopy using bis-benzimide (Hoechst 33258) staining. Briefly, the cells were seeded in 6-well plates at a density of 1×10^6 cells per well, followed by treatment with decursin for 48 h. After harvesting, the cells were washed twice with PBS and then stained with 200 μL of bis-benzimide (5 μg/mL) for 10 min at room temperature. Then, 10 μL of this suspension was placed on a glass slide and covered with a

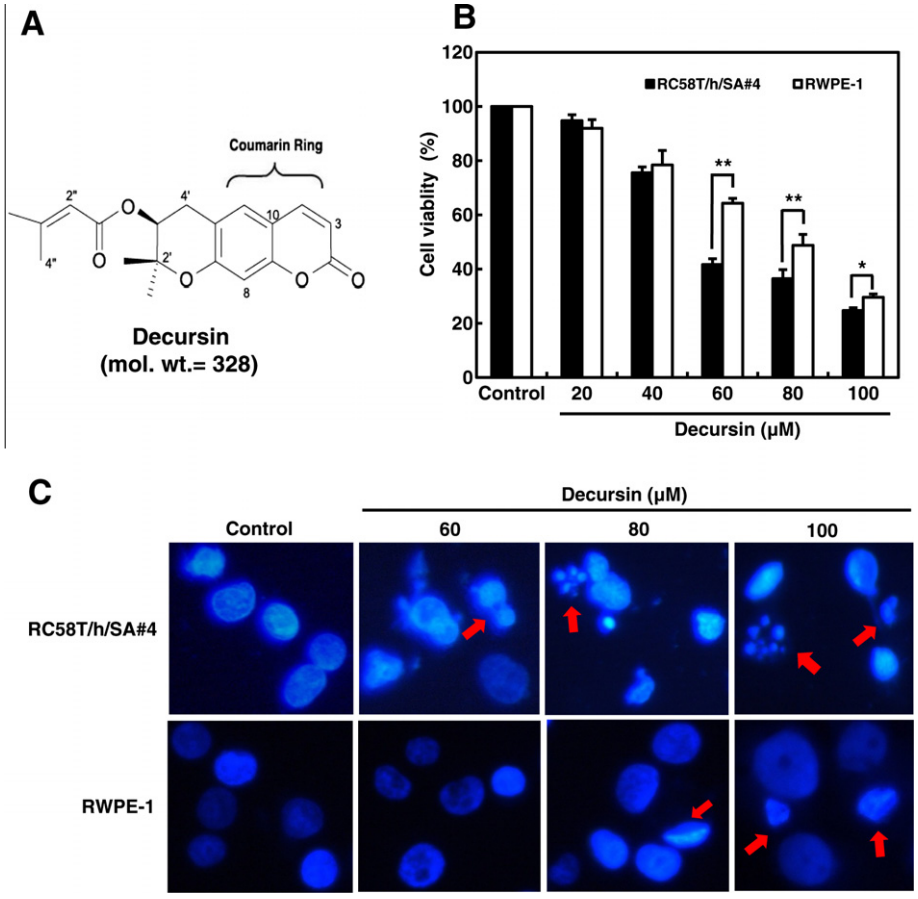

Fig. 1. Effect of decursin on the growth of prostate cancer cells. (A) Chemical structure of decursin isolated from roots of *A. gigas*. (B) Prostate cancer cells (RC58T/h/SA#4) and normal cells (RWPE-1) were treated with various concentrations of decursin for 48 h, and cell viability was determined by SRB assay. Data values are expressed as mean ± SD of triplicate determinations. Significant differences were compared with normal cells and prostate cancer cellsat $^*p < 0.05$ and $^{**}p < 0.01$ by Student's *t*-tests. (C) Representative images of nuclear condensation in response to decursin treatment as detected by Hoechst staining assay.