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Cytotoxicity of obacunone and obacunone glucoside in human prostate cancer cells involves Akt-mediated programmed cell death

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ABSTRACT

Obacunone and obacunone glucoside (OG) are naturally occurring triterpenoids commonly found in citrus and other plants of the Rutaceae family. The current study reports the mechanism of cytotoxicity of citrus-derived obacunone and OG on human androgen-dependent prostate cancer LNCaP cells. Both limonoids exhibited time- and dose-dependent inhibition of cell proliferation, with more than 60% inhibition of cell viability at 100 μM, after 24 and 48 h. Analysis of fragmentation of DNA, activity of caspase-3, and cytosolic cytochrome-c in the cells treated with limonoids provided evidence for activation of programmed cell death by limonoids. Treatment of LNCaP cells with obacunone and OG resulted in dose-dependent changes in expression of proteins responsible for the induction of programmed cell death through the intrinsic pathway and down-regulation of Akt, a key molecule in cell signaling pathways. In addition, obacunone and OG also negatively regulated an inflammation-associated transcription factor, androgen receptor, and prostate-specific antigen, and activated proteins related to the cell cycle, confirming the ability of limonoids to induce cytotoxicity through multiple pathways. The results of this study provided, for the first time, an evidence of the cytotoxicity of obacunone and OG in androgen-dependent human prostate cancer cells.

1. Introduction

An epidemiological study of Asian immigrants to the U.S. demonstrated the influence of diet and lifestyle on the incidence of prostate cancer (Crawford, 2003). Several cohort and case control studies have also demonstrated the association of diet with the incidence of prostate cancer (Chan et al., 2005; Key et al., 1997; Mills et al., 1989; Severson et al., 1989). These results were also supported by recent studies on the therapeutic benefits of dietary agents in prostate cancer (Venkateswaran and Klotz, 2010). These results impel us to improve our understanding of the role of natural dietary constituents in the prevention of prostate cancer.

Molecules that suppress androgen activity have important roles in the prevention and treatment of prostate cancer. Increased androgen activity causes up-regulation of cell-survival genes, leading to multiplication of prostate cancer cells (So et al., 2003). Suppression of androgen activity can occur through mitogen-activated protein kinases, protein kinase C, and Akt pathways, which cause phosphorylation of the androgen receptor (AR). AR phosphorylation increases AR transactivation, which affects the survival of prostate cancer cells (Edwards and Bartlett, 2005; Rochette-Egly, 2003). Androgen is also involved in *bcl-2*-mediated resistance to apoptosis (Berchem et al., 1995); therefore, inhibition of AR may also increase the sensitivity of cancer cells to *bcl-2*-mediated apoptotic cell death. Akt also termed protein kinase B, is a major cell-signaling molecule that regulates AR function and expression. In addition to its activity on AR, Akt also regulates cancer cell inhibition, through activation of death-inducing proteins, cell cycle arrest, induction of apoptosis, and inhibition of inflammatory responses. Therefore, suppression of Akt would be an ideal, multifaceted target for prevention or inhibition of multiplication of prostate cancer cells. The natural dietary compounds known to inhibit cancer cells through their

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effect on Akt include catechins from tea, indole-3-carbinols and brassinins from cruciferous vegetables, and the triterpene lupeol from fruits and vegetables (Kim et al., 2014; Rahman et al., 2004; Saleem et al., 2004; Shimizu and Weinstein, 2005).

We have previously demonstrated that citrus-derived bioactive molecules can inhibit the proliferation of colon, pancreatic, and breast cancer cells (Chidambara Murthy et al., 2011c; Jayaprakasha et al., 2008; Patil et al., 2009, 2010a; Tian et al., 2001). Among the different citrus limonoids examined, obacunone was the most potent inhibitor of cancer cell proliferation (Poulose et al., 2005; Somasundaram et al., 2007). In addition to their independent anti-proliferative ability, limonoids such as limonin and its glucoside also have an additive colon cancer inhibition effect when combined with curcumin (Chidambara Murthy et al., 2013). This inhibition activity results from their ability to induce programmed cell death in cancer cells (Poulose et al., 2005; Tian et al., 2001) and activate phase II enzymes in cancer-induced animals (Tanaka et al., 2001). Research at our laboratory also demonstrated the anti-aromatase activity of obacunone, along with other limonoids and glucosides, in human breast cancer cells (Kim et al., 2013). Obacunone and OG occur in very low concentrations in citrus fruits, from 7.2–60 ppm in different parts of the fruits (Ozaki et al., 1991, 1995). Therefore, in the current study, we used obacunone and OG isolated from seeds of white grapefruits in our laboratory. Recent studies from our laboratory and elsewhere showed that obacunone inhibits proliferation of different cancer cells and induces phase-II enzymes in carcinogen-challenged animals (Tanaka et al., 2001). Furthermore, our recent research on colon and pancreatic cancer cells demonstrated the ability of obacunone to induce programmed cell death and inhibit inflammation (Chidambara Murthy et al., 2011b). Another interesting attribute of citrus limonoids is their ability to not interfere with clinical chemotherapeutic agents, as tested against camptothecin and cyclophosphamide in different breast cancer cell lines (Somasundaram et al., 2012). This property makes limonoids suitable for clinical use in adjuvant or neoadjuvent therapy. Despite these favorable attributes, very little is known regarding their effects on hormone-dependent human cancer cells; therefore, here we examined the role of obacunone and obacunone glucoside on cell signaling-related pathways in LNCaP cells, for the first time.

2. Materials and methods

2.1. Reagents

RPMI-1640 medium and cell culture chemicals were obtained from Hyclone (Logan, UT, USA); antibiotics and trypsin EDTA were obtained from Mediatech Inc. (Herndon, VA, USA). The

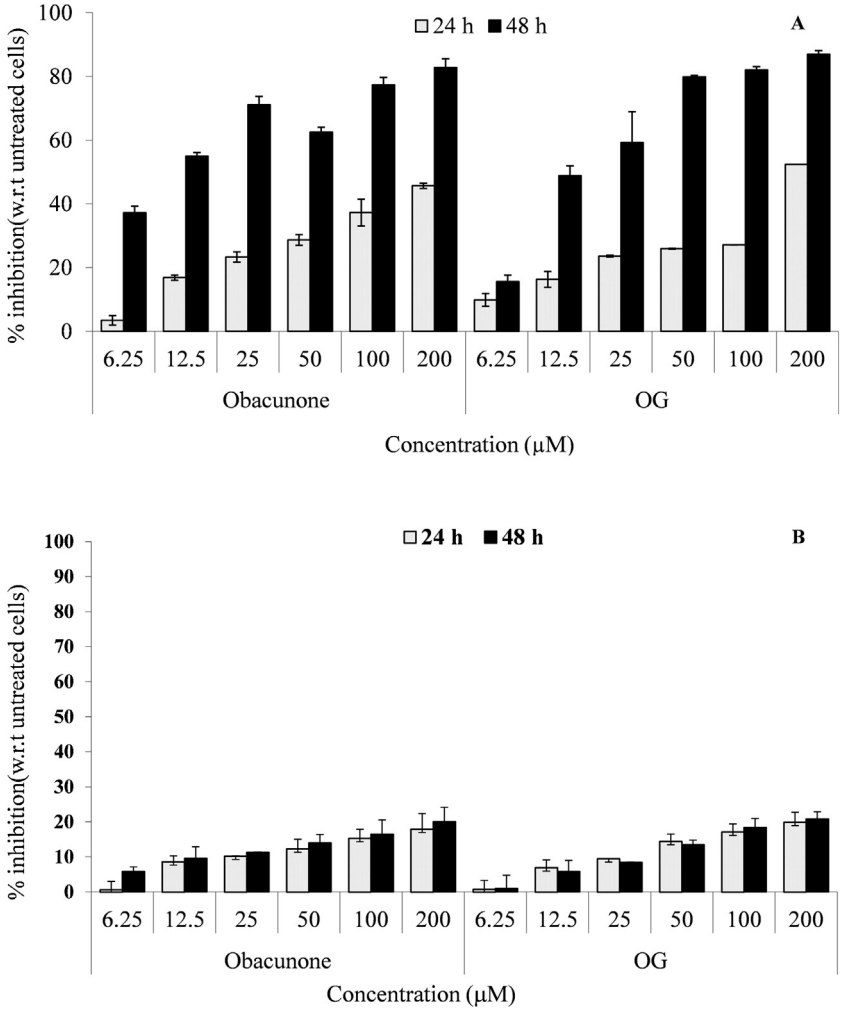

Fig. 1. Effect of obacunone and OG on proliferation of LNCaP prostate cancer cells. (A) Inhibition of cell proliferation of LNCaP cells as measured by MTT assays. Results are expressed as mean ± S.D from three biological replicates ($n = 9$). Values are significant at $P < 0.05$ compared to control at all the concentrations and time points, except obacunone treatment for 24 h at 6.25 μM. (B) Inhibition of RWPE-1 cell proliferation measured by MTT assay. Viability of RWPE-1 cells was measured by MTT assay and results are expressed as mean ± S.D from three biological replicates ($n = 8–9$).