Benzyldihydroxyoctenone, a Novel Nonsteroidal Antiandrogen, Shows Differential Apoptotic Induction in Prostate Cancer Cells in Response to Their Androgen Responsiveness

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The molecular mechanisms of apoptotic induction by benzyldihydroxyoctenone (BDH), a nonsteroidal antiandrogen, isolated from the culture broth of *Streptomyces* sp., have been previously published in prostate cancer LNCaP cells. Apoptotic induction of BDH-treated LNCaP cells was associated with downregulation of Bcl-xL that caused, in turn, cytochrome c release from mitochondria, and activation of procaspases and specific proteolytic cleavage of poly(ADP-ribose) polymerase (PARP). The purpose of the present study was to investigate the patterns of apoptotic induction by BDH in non-prostate, ovarian cancer PA-1 (androgen-independent and -insensitive) cells and prostate cancer cells with different androgen responsiveness, such as C4-2 (androgen-independent and -insensitive), 22Rv1 (androgen-dependent and -low sensitive), and LNCaP (androgen-dependent and -high sensitive) cells. We found that BDH-treated LNCaP cell proliferation was significantly inhibited in a time-dependent manner and induced apoptosis via downregulation of the androgen receptor (AR) and prostate-specific antigen (PSA), as well as antiapoptotic Bcl-xL protein. However, the levels of BDH-mediated apoptotic induction and growth inhibition in 22Rv1 cells were apparently lower than those of LNCaP cells. In contrast, the induction of apoptosis and antiproliferative effect in BDH-treated non-prostate cancer PA-1 and hormone refractory C4-2 cells were not detectable and marginal, respectively. Therefore, BDH-mediated differential apoptotic induction and growth inhibition in a cell type seem to be obviously dependent on its androgen responsiveness; primarily on androgen-dependency, and then on androgen-sensitivity.

**Key words:** Benzyldihydroxyoctenone, antiandrogen, apoptosis, androgen receptor, prostate cancer

Apoptosis, a programmed cell death, is a fundamental process for normal development and homeostasis of multicellular organisms, and is regulated by expression of multiple genes [4, 17]. Apoptotic signaling event occurs through multiple independent pathways that are initiated by various extra- and intracellular factors [19]. Activation of procaspase to caspase plays a critical role in the execution of apoptotic signaling pathways [13, 19]. Furthermore, many lines of evidence demonstrate that Bcl-2-related proteins play an important role in either inhibition or promotion of apoptosis via regulating the release of cytochrome c from the mitochondria. Thus, the Bcl-2 family of proteins constitutes a critical intracellular checkpoint of apoptosis [22]. The Bcl-2 family can be divided into two main subclasses: (I) the antiapoptotic members: Bcl-2, Bcl-xL, and Bcl-w; (II) the proapoptotic members: Bax, Bak, Bid, and Puma [5]. In general, apoptosis may be mediated through either downregulation of antiapoptotic proteins or upregulation of proapoptotic proteins, and results in increase of the Bax/Bcl-2 ratio.

The relationship between deregulation of apoptosis and cancer formation has been emphasized, with increasing evidence suggesting that neoplastic transformations involve the alteration of normal apoptotic pathways. Recently, considerable attention has been focused on the discovery of small chemicals, including natural products, which exert their antitumor effects by apoptotic induction in various cancer cells [6, 7, 10, 11, 14–16].

Prostate cancer is the most frequently diagnosed cancer and is the leading cause of cancer death in men in the US, with an estimated 217,730 new cases and 32,050 deaths in 2010 according to the latest estimation of the American Society of Clinical Oncology Prostate Cancer Statistics (http://...
Despite the initial efficacy of androgen deprivation therapy, the advanced prostate cancer patients eventually develop resistance to this therapy and progress to hormone-refractory prostate cancer (HRPC), for which there is no curative therapy [3]. Therefore, novel targeted therapeutic approaches have to be developed for the treatment of HRPC patients [2].

Androgen exerts its biological effects by binding to the androgen receptor (AR). Upon binding to the AR, androgen activates the AR through phosphorylation, dimerization, and nuclear translocation, which in turn interacts with androgen response elements (ARE) in the promoter of target genes including prostate-specific antigen (PSA), regulating the transcription of target genes. Therefore, PSA is a clinically important marker used to monitor the diagnosis, treatment response, prognosis, and progression in patients with prostate cancer [9].

The human prostate cancer LNCaP cells are useful for investigating the molecular mechanisms responsible for the changes in androgen sensitivity. Recently, several human prostate cancer cells with different androgen responsiveness were generated and characterized. 22Rv1 cells are derived from a xenograft that was serially propagated in mice after castration-induced regression and relapse of the parental, androgen-dependent CWR22 xenograft [18]. Therefore, 22Rv1 cells are less sensitive to androgen-mediated growth stimulation and PSA production than LNCaP cells. In contrast to 22Rv1 and LNCaP cells, C4-2 cells are androgen refractory, highly tumorigenic, and metastatic in a castrated host [20, 21]. Therefore, 22Rv1 and C4-2 cells could be used as valuable models for anticancer drug screening, particularly screening for a novel antiandrogen.

Previously, we have published molecular mechanisms of apoptotic induction by BDH (Fig. 1A) in HeLa and LNCaP cells [8, 12]. Apoptotic induction of BDH-treated LNCaP cells was associated with downregulation of Bcl-xL, which caused, in turn, cytochrome c release from mitochondria, and activation of procaspase-3, -9 and specific proteolytic cleavage of PARP [12]. The current study was aimed to investigate the patterns of apoptotic induction by BDH, an androgen antagonist, in non-prostate PA-1 and prostate cancer cells with different androgen responsiveness, such as C4-2, 22Rv1, and LNCaP cells (Table 1).

To examine the antiproliferative effect of 30 µM of BDH in the 4 cell lines described above, real-time Cell Electronic Sensing (RT-CES) was performed. RT-CES (ACEA Biosciences, San Diego, CA, USA) is a novel cell-based assay system to monitor cellular events by measuring the electronic impedance of sensor electrodes integrated on the bottom of microtiter E-Plates. Based on measured impedance, a dimensionless parameter, cell index, is derived and reported to provide quantitative information about the biological status of the cell viability.

As shown in Fig. 1B, the cell index of the androgen-independent group after BDH treatment was either almost unchanged (C4-2) or even apparently increased in a time-dependent manner (PA-1). In contrast, that of the androgen-dependent group, including 22Rv1 and LNCaP cells, was significantly decreased. Moreover, the BDH-mediated antiproliferative effect of each group seemed to be relatively amplified, if cells are more sensitive to androgen. Therefore, androgen dependency and sensitivity are responsible for BDH-mediated growth inhibition in prostate cancer cells tested so far.

To investigate the apoptotic induction of BDH in a target cell line, a TUNEL assay was conducted to examine

**Table 1.** The characteristics of cancer cells used in the current study.

<table>
<thead>
<tr>
<th>Cells</th>
<th>Sensitivity and dependency on androgen</th>
<th>Apoptosis</th>
<th>References</th>
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<tbody>
<tr>
<td>PA-1</td>
<td>Inensitive</td>
<td>Independent</td>
<td>--</td>
</tr>
<tr>
<td>C4-2</td>
<td>Sensitive</td>
<td>Independent</td>
<td>+/-</td>
</tr>
<tr>
<td>22Rv1</td>
<td>Low-sensitive</td>
<td>Dependent</td>
<td>+</td>
</tr>
<tr>
<td>LNCap</td>
<td>High-sensitive</td>
<td>Dependent</td>
<td>++</td>
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DNA fragmentation in the nuclei of LNCaP, 22Rv1, and C4-2 cells. As shown in Fig. 2A, the apoptotic induction was identified in LNCaP and 22Rv1 cells treated with 30 μM of BDH for 48 h. However, BDH-mediated apoptotic induction was not shown in C4-2 cells. To determine the molecular mechanisms involved in mediation of BDH-induced cell death in LNCaP and 22Rv1 cells, caspase activation and cleavage of PARP were tested using Western blotting. As shown in Fig. 2B, caspase activation and cleavage of PARP, as well as downregulation of AR and Bcl-xL, were detected in BDH-treated LNCaP and 22Rv1 cells, whereas apoptotic induction in PA-1 and C4-2 cells was not shown. Many lines of evidence demonstrate that Bcl-2-related proteins play an important role in either inhibition or promotion of apoptosis [1]. Our results suggest that BDH-induced apoptosis may be mediated through downregulation of antiapoptotic proteins, and resulted in increase of the Bax/Bcl-2 ratio in LNCaP and 22Rv1 cells.

Furthermore, we found that BDH significantly inhibited the expression levels of AR genes in LNCaP and 22Rv1, and marginally in C4-2 cells, which showed no apoptotic induction in TUNEL assay and Western blot analysis (Fig. 2).

In this context, we examined the expression level of PSA, a primary AR downstream gene, in LNCaP, 22Rv1, and C4-2 cells using RT-PCR and quantitative real-time PCR, to investigate whether induction of apoptosis of BDH-treated androgen-dependent cells is mainly due to downregulation of AR activation. As shown in Fig. 3, we found that expression of PSA was significantly inhibited in BDH-treated LNCaP (88%) and 22Rv1 (67%) cells (Fig. 3B), whereas almost no change in C4-2 cells was seen (Fig. 3A). Therefore, BDH has been shown to be an androgen antagonist, suggesting however that BDH could be a potent agent only for treatment of androgen-dependent and -sensitive prostate cancer cells.

In conclusion, BDH exhibits an antiproliferative effect by induction of apoptosis associated with downregulation of Bcl-xL, and activation of procaspases, as well as cleavage of PARP in androgen-dependent LNCaP and 22Rv1 cells. However, the levels of BDH-mediated apoptotic induction and growth inhibition in 22Rv1 cells were apparently lower than those of LNCaP cells. Furthermore, the apoptotic induction and antiproliferative effect in BDH-treated non-prostate cancer PA-1 and hormone refractory C4-2 cells.
were non-detectable and marginal, respectively. Therefore, BDH-mediated apoptotic induction and growth inhibition seem to be associated primarily with androgen-dependency, and then with androgen-sensitivity of the cells.

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REFERENCES


