Bioorganic & Medicinal Chemistry Letters 21 (2011) 1347–1349

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# HY251, a novel decahydrocyclopenta[a]indene analog, from *Aralia continentalis* induces apoptosis via down-regulation of *AR* expression in human prostate cancer LNCaP cells

Ha Lim Oh [a], Chul-Hoon Lee [b,*]

[a] *Institute of Medical Science, College of Medicine, Hanyang University, Seoul 133-791, Republic of Korea*
[b] *Department of Pharmacy, College of Pharmacy, Hanyang University, Kyeonggi-do 426-791, Republic of Korea*

ARTICLE INFO

*Article history:*
Received 3 December 2010
Revised 11 January 2011
Accepted 12 January 2011
Available online 15 January 2011

*Keywords:*
*Aralia continentalis*
Apoptotic induction
Androgen receptor
LNCaP cells

ABSTRACT

In the course of screening for a novel anticancer drug candidate, we previously isolated HY251 with the molecular structure of 3-propyl-2-vinyl-1,2,3,3a,3b,6,7,7a,8,8a-decahydrocyclopenta[a]indene-3,3a,7a,8a-tetraol from the roots of *Aralia continentalis*. The current study was designed to evaluate the detailed mechanisms of apoptotic induction of HY251 in androgen-sensitive prostate cancer LNCaP cells. TUNEL assay and Western blot analysis revealed an appreciable apoptotic induction in LNCaP cells treated with 95 μM of HY251 for 24 h. This apoptotic induction is also associated with cytochrome c release from mitochondria which, in turn, resulted in the activation of caspase-9 and -3, and the cleavage of poly(-ADP-ribose) polymerase (PARP). Moreover, we found that HY251 significantly inhibited the expression levels of androgen receptor (AR) and prostate-specific antigen (PSA) in a time-dependent manner, as well as abrogated up-regulation of *AR* and *PSA* genes with and without androgen. Therefore, we suggest that HY251, a novel androgen antagonist, may be a potent cancer chemotherapeutic candidate for the treatment of both androgen-sensitive and hormone-refractory prostate cancer.



Apoptosis, a programmed cell death, and cell proliferation have been characterized as fundamental cellular events to maintain the physiological balance and tissue homeostasis of the organism. Therefore, an imbalance between cell proliferation and death through deregulated cell cycle progression and impaired apoptotic induction is involved in neoplastic cancer formation.[1] Apoptosis can be induced through either the stimulation of the death receptors, such as Fas and TNFR1 or the release of signal factors, such as cytochrome c, from mitochondria.[2] Recently, considerable attention has been focused on the discovery of small chemicals, including natural products, which exert their anticancer effects by apoptotic induction in various cancer cells.[3–5]

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 American Cancer Society. 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.[6] Therefore, novel targeted therapeutic approaches have to be developed for the treatment of HRPC patients.[7]

Androgen, such as dihydrotestosterone (DHT), exerts its biological effects by binding to AR. Upon binding to AR, androgen activates AR through phosphorylation, dimerization and nuclear translocation, which, in turn, interacts with androgen response elements (ARE) in the promoter of target genes including PSA, regulating the transcription of target genes. Therefore, PSA is a clinically important marker used to monitor diagnosis, treatment response, prognosis, and progression in patients with prostate cancer.[8] Studies on the progression of prostate cancer have indicated that an increase in AR at transcriptional and translational level is necessary to convert prostate cancer from a hormone-sensitive to a hormone refractory state,[9] and that over-expressed AR linked to p21 silencing may be responsible for androgen independence and resistance to apoptosis.[10] Therefore, AR is a key target for the treatment of both early stage prostate cancer and HRPC, and the discovery of AR antagonist should be an important rational approach for the successful treatment of HRPC.

The medicinal herb *Aralia continentalis* of the Araliaceae family is spread widely throughout northeast Asian countries. Many constituents from its root extracts have been isolated as active components for antioxidant, anti-inflammatory, analgesic, sedative, antifungal, anti-thrombotic, and antitumor agents.[11–13] In the course of screening for novel modulators on apoptotic induction and/or cell cycle progression as anticancer drug candidates, as published previously, we found HY251, a novel decahydrocyclopenta[a]indene tetraol (Fig. 1), and its decahydrofluorene analog,

* Corresponding author. Tel.: +82 31 400 5801.
*E-mail address:* chhlee@hanyang.ac.kr (C.-H. Lee).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.01.045

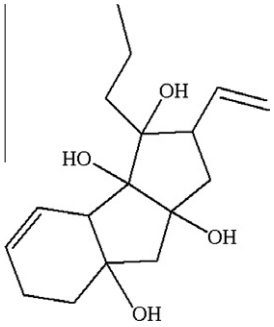


**Figure 1.** The chemical structure and nomenclature of HY251, 3-propyl-2-vinyl-1,2,3,3a,3b,6,7,7a,8,8a-decahydrocyclopenta[a]indene-3,3a,7a,8a-tetraol.

HY253, from the roots of *A. continentalis* as cell cycle inhibitor and apoptotic inducer in HeLa and A549 cells.[14–16] In the current study, we report, for the first time, the molecular mechanisms of HY251 on the apoptotic induction in androgen-sensitive prostate cancer LNCaP cells.

To investigate the effects of HY251 on apoptotic induction in LNCaP cells, we measured DNA fragmentation in the nuclei of LNCaP cells treated with 95 μM of HY251, the $IC_{50}$ value obtained from MTT assay (data not shown), using TUNEL assay. As shown in Figure 2, the induction of apoptosis was identified via peak shift to the right direction due to DNA fragmentation in LNCaP cells treated with 95 μM of HY251 for 24 h.

To determine whether apoptosis-related proteins are involved in the mediation of HY251-induced cell death in LNCaP cells, we examined caspases activation and cleavage of PARP by Western blot analysis. As shown in Figure 3, HY251 induced the proteolytic cleavage of inactive procaspase-9, and -3 into active caspase-9, and -3, respectively. Furthermore, one of the substrates for effector caspases, such as caspase-3, during apoptosis is PARP, an enzyme that appears to be involved in DNA repair. Therefore, the cleavage of PARP was used as an indicator of apoptotic induction in response to drug treatment, which became obvious after 12 h of HY251(95 μM) treatment.

In addition, to examine the mitochondria-mediated intrinsic pathway involved in HY251-induced apoptosis, we analyzed the cytochrome c release from mitochondria to cytosol using Western blots. As shown in Figure 3, cytochrome c release was observed significantly in LNCaP cells treated with 95 μM of HY251 for 24 h.

However, in contrast to the apoptotic induction of HY253 in HeLa cells,[15] we could not demonstrate the significant changes in the level of pro-apoptotic and anti-apoptotic Bcl-2 proteins in HY251-treated LNCaP cells tested so far (data not shown). Therefore, further studies on protein–protein interactions of pro- and anti-apoptotic proteins and their translocation to mitochondria are needed to confirm the detailed molecular mechanisms of HY251 on apoptotic induction in LNCaP cells.

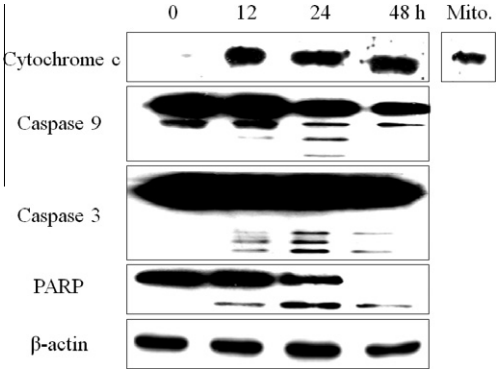


**Figure 3.** Effects of HY251 on cytochrome c release from mitochondria, and activation of caspase-9 and -3, as well as cleavage of PARP. Cells pretreated with 95 μM of HY251 for different lengths of time were washed with PBS, and Western blot analysis performed. β-actin was used as the loading control.

Moreover, to investigate whether induction of apoptosis by HY251 is due to the down-regulation of *AR* gene, Western blot analysis and quantitative RT-PCR were conducted in HY251-treated LNCaP cells.[17] As shown in Figure 4, we found that HY251 significantly inhibited the expression levels of AR and PSA proteins in a time-dependent manner, and abrogated up-regulation of *AR* and *PSA* genes in LNCaP cells with and without DHT (1.0 nM).

The androgen-mediated AR signaling plays a pivotal role in both the development and maintenance of the normal prostate as well as in the initiation and progression of prostate cancer.[18] AR belongs to the nuclear steroid hormone receptor superfamily and is the essential mediator of androgen action.[19] Upon binding to the native ligand, such as DHT, AR dissociates from the chaperone proteins and rapidly translocates to the nucleus where it binds to ARE and activates target genes including PSA.[20]

Recently, considerable attention has been focused on the discovery of AR antagonists which exert their anticancer effects by suppression of the androgen-dependent cell proliferation and apoptotic induction via down-regulation of pivotal survival factors and anti-apoptotic proteins, such as PSA and Bcl-2/Bcl-XL, respectively.[21,22]

In conclusion, we found that HY251 significantly inhibited the expression levels of AR and PSA proteins in a time-dependent manner, and abrogated up-regulation of *AR* and *PSA* genes in LNCaP cells. As shown in Figure 5, HY251 could be a potent candidate for the treatment of both androgen-dependent LNCaP ($IC_{50}$: 94 μM) and hormone-refractory prostate cancer C4-2 cells ($IC_{50}$: 198.6 μM), in contrast to Fluthamide, an anti-androgen drug, which shows no anti-proliferative effect on C4-2 cells. However, more detailed molecular mechanisms underlying apoptotic induction in HY251-treated LNCaP cells via down-regulation of *AR* gene by HY251 should be identified in a future.

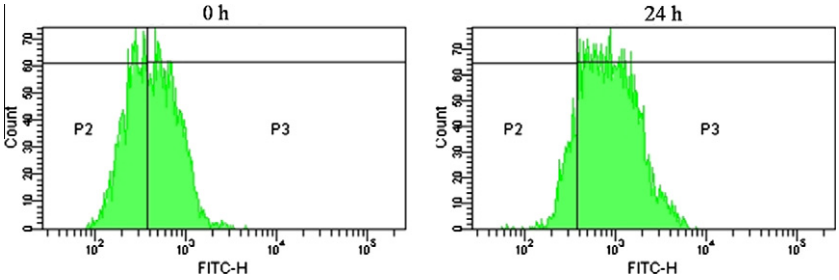


**Figure 2.** The induction of apoptosis by HY251 in LNCaP cells, as determined by TUNEL assay. The cells were treated with 95 μM of HY251 for 24 h, and then stained with d-UTP FITC and Propidium Iodide (PI) in the dark and analyzed using a FACS Canto II with BD FACSDiva Software v6.1.3.

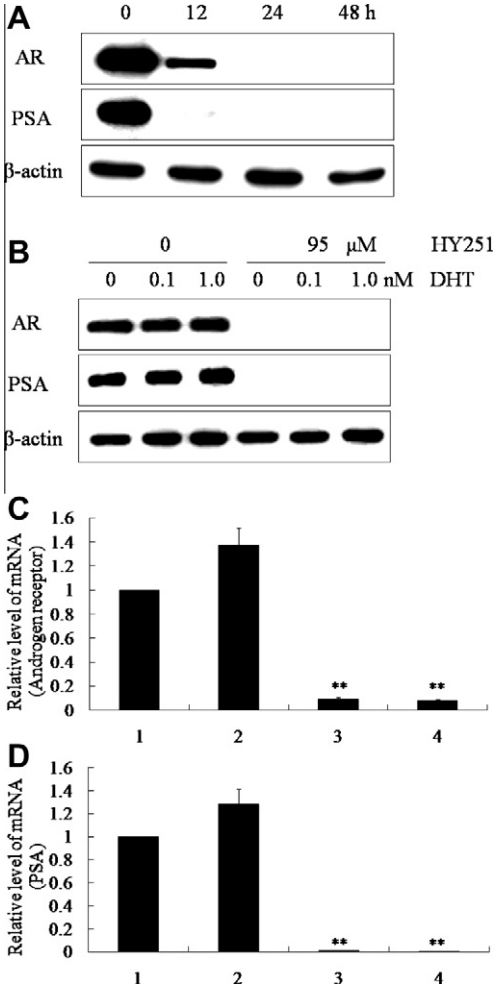


**Figure 4.** (A) HY251 significantly inhibited the expression of AR and PSA at protein levels and the secretion of PSA in LNCaP cells. (B) HY251 (95 μM) abrogated the induction of *AR* and *PSA* expression induced by 0.1 and 1 nM of DHT treatment for 2 h in LNCaP cells. The quantitative RT-PCR demonstrated that HY251 significantly down-regulated the transcription of *AR* (C) and *PSA* (D) in LNCaP cells with and without DHT (1.0 nM). Data are means ± SD of three separate experiments. 1: control, 2: DHT (1.0 nM), 3: DHT (1.0 nM) + HY251 (95 μM), 4: HY251 (95 μM). $^{**}P$ <0.01.

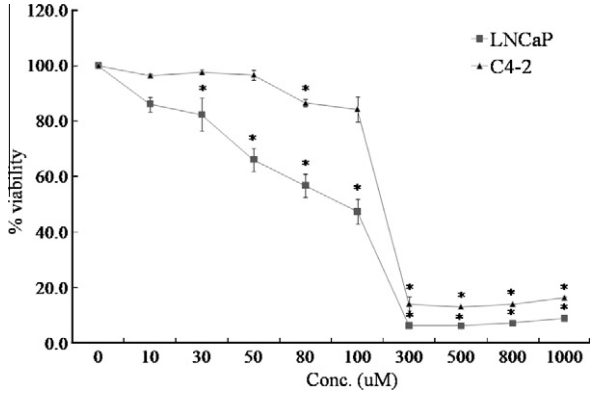


**Figure 5.** Effect of HY251 on proliferation of androgen-dependent LNCaP and HRPC C4-2 cells in a dose-dependent manner, as determined by MTT assay for 24 h. Results represent the mean ± SD of three independent experiments. $^{*}P$ <0.05.

## Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (Nos. 313-2008-2-E00117 and 2010 0012838).

## References and notes

1. Bold, R. J.; Termuhlen, P. M.; McConkey, D. J. *Surg. Oncol.* **1997**, *6*, 133.
2. Fulda, S.; Debatin, K. M. *Oncogene* **2006**, *25*, 4798.
3. Kim, M. K.; Min, J.; Choi, B. Y.; Lim, H.; Cho, Y. H.; Lee, C. H. *J. Microbiol. Biotechnol.* **2007**, *17*, 1712.
4. Kang, J.; Lee, D. K.; Lee, C. H. *J. Microbiol. Biotechnol.* **2010**, *20*, 433.
5. Lim, H.; Oh, H. L.; Lee, C. H. *J. Microbiol. Biotechnol.* **2006**, *16*, 1399.
6. Bracarda, S.; de Cobelli, O.; Greco, C.; Prayer-Galetti, T.; Valdagni, R.; Gatta, G.; de Braud, F.; Bartsch, G. *Crit. Rev. Oncol. Hematol.* **2005**, *56*, 379.
7. Bhuiyan, M. M.; Li, Y.; Banerjee, S.; Ahmed, F.; Wang, Z.; Ali, S.; Sarkar, F. H. *Cancer Res.* **2006**, *66*, 10064.
8. Lieberman, R. *Am. J. Ther.* **2004**, *11*, 501.
9. Chen, C. D.; Welsbie, D. S.; Tran, C.; Baek, S. H.; Chen, R.; Vessella, R.; Rosenfeld, M. G.; Sawyers, C. L. *Nat. Med.* **2004**, *10*, 33.
10. Wang, L. G.; Ossowski, L.; Ferrari, A. C. *Cancer Res.* **2001**, *61*, 7544.
11. Han, B. H.; Han, Y. N.; Han, K. A.; Park, M. H.; Lee, E. O. *Arch. Pharm. Res.* **1983**, *6*, 17.
12. Han, B. H.; Park, M. H.; Han, Y. N.; Manalo, J. B. *Arch. Pharm. Res.* **1983**, *6*, 75.
13. Ryu, S. Y. *Arch. Pharm. Res.* **1996**, *19*, 77.
14. Oh, H. L.; Lim, H.; Cho, Y. H.; Koh, H. C.; Kim, H.; Lim, Y.; Lee, C. H. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 959.
15. Oh, H. L.; Lim, H.; Park, Y.; Lim, Y.; Koh, H. C.; Cho, Y. H.; Lee, C. H. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 797.
16. Oh, H. L.; Lee, D. K.; Lim, H.; Lee, C. H. *J. Ethnopharmacol.* **2010**, *129*, 135.
17. *The quantitative RT-PCR*: LNCaP cells were cultured in RPMI 1640 with 10% FBS for 48 h. Cells were then treated with 95 μM of HY251 in 100% DMSO for 24 h followed by treatment with and without DHT (0.1 and 1 nM) for 2 h. After treatment, total RNA was extracted from the cells using RNeasy Mini Kit (Qiagen, Valencia, USA). Total RNA (2 μg) from each sample was subjected to reverse transcription using the SuperScript First-Strand cDNA Synthesis Kit (Invitrogen, USA) and the cDNAs were subjected to real-time PCR analysis for *AR* and *PSA* expression. Real-time PCR reactions were carried out in iCycler iQ (Bio-Rad, USA). The specific primers for *AR* were as follows: 5′-GTGGACGACCAGATGGCTGT-3′ and 5′-GAAGAGTAGCAGTGCTTTCATGC-3′. The primers for *PSA* were as follows: 5′-TTGTCTTCCTCACCCTGTCC-3′ and 5′-CATCAGGAACAAAAGCGTGA-3′. The primers for *GADPH* were as follows: 5′-GTGGGGCGCCCCAGGCACCAGGGC-3′ and 5′-CTCCTTAATCTCACGCACGAT TTC-3′. PCR amplification efficiency and linearity for each gene including targeted and control genes were tested. The relative quantization was calculated using the comparative threshold cycle method.[23] House-keeping gene *GAPDH* was used to confirm the homogeneity of the DNA products.
18. Chang, C.; Saltzman, A.; Yeh, S.; Young, W.; Keller, E.; Lee, H. J.; Wang, C.; Mizokami, A. *Crit. Rev. Eukaryot. Gene Expr.* **1995**, *5*, 97.
19. Gobinet, J.; Poujol, N.; Sultan, C. H. *Mol. Cell. Endocrinol.* **2002**, *198*, 15.
20. Huggins, C.; Hodges, C. V. *J. Urol.* **2002**, *168*, 9.
21. Moon, H. S.; Lim, H.; Moon, S.; Oh, H. L.; Kim, Y.; Kim, M. K.; Lee, C. H. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 742.
22. Marrocco, D. L.; Tilley, W. D.; Bianco-Miotto, T.; Evdokiou, A.; Scher, H. I.; Rifkind, R. A.; Marks, P. A.; Richon, V. M.; Butler, L. M. *Mol. Cancer Ther.* **2007**, *6*, 51.
23. Kenneth, J. L.; Thomas, D. S. *Methods* **2001**, *25*, 402.