Induction of apoptosis and cell cycle arrest by a chalcone panduratin A isolated from *Kaempferia pandurata* in androgen-independent human prostate cancer cells PC3 and DU145

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Because of unsatisfactory treatment options for prostate cancer (CaP) there is a need to develop novel preventive approaches for this malignancy. One such strategy is through chemoprevention by the use of non-toxic dietary substances and botanical products. We have shown previously that panduratin A isolated from the extract of *Kaempferia pandurata* (*Zingiberaceae*) is a strong inhibitor of cyclooxygenase-2 in RAW264.7 cells and induces apoptosis in HT-29 cells. In the present study, we provide evidence that panduratin A treatment to androgen-independent human CaP cells PC3 and DU145 result in a time and dose-dependent inhibition of cell growth with an IC50 of 13.5–14 μM and no to little effect on normal human prostate epithelial cells. To define the mechanism of these anti-proliferative effects of panduratin A, we determined its effect on critical molecular events known to regulate the cell cycle and the apoptotic machinery. Annexin V/propidium iodide staining provided the evidence for the induction of apoptosis which was further confirmed by the observation of cleavage of poly (ADP-ribose) polymerase and degradation of acinus. Panduratin A treatment to cells was found to result in inhibition of procaspases 9, 8, 6 and 3 with significant increase in the caspases (8) and induces apoptosis in human colon cancer HT-29 cells. In the present study, we demonstrate antiproliferative and proapoptotic effect of panduratin A in human androgen-independent CaP cells, PC3 and DU145, and delineate the mechanism of this effect.

Introduction

Prostate cancer (CaP) is the second most prevalent malignancy and the second leading cause of cancer-related deaths among men in the USA with similar trend in many western countries (1). Clinically, CaP is usually diagnosed in men over 50 years of age; with increasing life expectancy the incidence of CaP is likely to increase worldwide (1–2). Thus, there is a promising opportunity for its intervention using cancer chemopreventive compounds that can prevent or slow the progression of this disease (3–4). For a variety of reasons naturally occurring botanicals and dietary substances are gaining increasing attention as cancer chemopreventive agents. Important for CaP chemoprevention is the fact that in recent years, the use of dietary substances and botanical products is showing sustained increase by CaP patients. In CaP a fine balance between cell proliferation and apoptotic death is lost which contributes to increase in cellular mass and tumor progression. In this regard, for CaP chemoprevention at the present time there is considerable emphasis in identifying novel botanicals that selectively induces apoptosis and growth arrest of CaP cells without producing cytotoxic effects on normal cells.

The rhizome of *Kaempferia pandurata* Roxb. (*Zingiberaceae*), a herb cultivated in some tropical countries including Indonesia and Thailand, has been used as a condiment and folk medicine for treatment of various ailments, including colic disorder, fungal infections, dry cough, rheumatism and muscular pains (5–6). Since chalcones are natural products which have been reported to possess a variety of biological properties, including anti-inflammatory, analgesic and antioxidant activities (7). We recently isolated panduratin A (Figure 1), a cyclohexenyl chalcone derivative, from the methanolic extract of *K.pandurata* and reported that it exhibits strong COX-2 inhibitory activity in mouse peritoneal macrophages (8) and induces apoptosis in human colon cancer HT-29 cells (9). In the present study, we demonstrate antiproliferative and proapoptotic effect of panduratin A in human androgen-independent CaP cells, PC3 and DU145, and delineate the mechanism of this effect.
Efficacy of panduratin A against prostate cancer cells

**Materials and methods**

**Plant material and chemicals**

Anti-caspases, anti-FADD, anti-Fas, anti-Fasl, anti-TRAIL, anti-Bid and anti-p27kip1 antibodies were procured from Santa Cruz Biotechnology, (Santa Cruz, CA). Anti-cyclins, anti-Bcl-2, anti-Bax, anti-cdk2, anti-p-cdk2, anti-cdk25C and anti-p21WAF1/Cip1 antibodies were procured from Cell Signaling Technology (Beverly, MA). Anti-PAPP 116 and anti-cdk2 antibodies were procured from Upstate Biotechnology (Lake Placid, NY). Anti-PARP p85 antibody was procured from Promega (Madison, WI). Anti-cdk4 and anti-cdk6 antibodies were procured from Biosource International (Camarillo, CA). The active compound identified as panduratin A was isolated from the methanolic extract of *K.pandurata* (Zingiberaceae) as described previously (8). Panduratin A (Figure 1) was identified by comparison of the spectral (1'H-NMR, 13C-NMR and FAB-MS) properties with published data (6,7,10). Copies of the original spectra can be obtained on request from the corresponding author. Optical rotation was measured with a Perkin-Elmer 241 polarimeter as [α]21.5-gauge needle to break up the cell aggregates. The lysate was cleared by centrifugation at 13 000 × g for 20 min. The cells were then chilled over ice for 10 min and stained with PI (50 μg/ml final concentration) for 1 h for analysis by flow cytometry. Flow cytometry was performed with a FACScan (Becton Dickinson, Germany). A minimum of 10 000 cells/sample were collected, and the DNA histograms were further analyzed using ModFitLT software (Verity Software House, Topsham, ME, USA) for cell cycle analysis.

**Protein extraction and western blot analysis**

Following treatment of cells with panduratin A, the medium was aspirated and the cells were washed with cold PBS (10 mmol/l (pH 7.45)). The cells were then incubated in ice-cold lysis buffer (50 mmol/l Tris–HCl, 150 mmol/l NaCl, 1 mmol/l EGTA, 1 mmol/l EDTA, 20 mmol/l NaF, 100 mmol/l Na3VO4, 0.5% NP40, 1% Triton X-100 and 1 mmol/l phenylmethylsulfonyl fluoride (pH 7.4)) with freshly added protease inhibitor cocktail (protease inhibitor cocktail set III, Calbiochem, La Jolla, CA) over ice for 20 min. The cells were scraped and the lysate was collected in a microfuge tube and passed through a 21.5-gauge needle to break up the cell aggregates. The lysate was cleared by centrifugation at 13 000 × g for 15 min at 4°C, and the supernatant (total cell lysate) was stored, aliquoted and used on the day of preparation or immediately stored at −80°C for use at a later time. For western blot analysis, 40 μg of the protein was resolved over 12% polyacrylamide gels and transferred onto a nitrocellulose membrane. The non-specific sites on blots were blocked by incubating in blocking buffer (5% non-fat dry milk, 1% Tween-20 in 20 mmol/l TBS (pH 7.6)) for 1 h at room temperature, incubated with appropriated monoclonal or polyclonal primary antibody in blocking buffer for 90 min to overnight at 4°C, followed by incubation with anti-mouse or anti-rabbit secondary antibody horseradish peroxidase conjugate and detected by chemiluminescence and autoradiography using Hyperfilm obtained from Amersham Biosciences. Densitometric measurement of the bands in western blot analysis were done using digitalized scientific software program UN-SCAN-IT purchased from Silk Science Corporation (Orem, UT).

**Reverse-transcription-polymerase chain reaction (RT–PCR)**

Total RNA was isolated from the cells using a commercial RNeasy kit (Qiagen, Valencia, CA), and RNA concentration was measured spectrophotometrically at 260 nm. Total RNA (1 μg) was subjected to RT–PCR using one-step RT–PCR premix kit (Promega) containing all components required to synthesize cDNA and to perform the PCR, and specific primers for p27kip1 and GAPDH as a loading control: p27kip1 (size of PCR product: 523 bp): sense 5'-GAA CCG CCA TTT GGG GAA CC-3', antisense 5'-TAA CCC GGG ACT TGG AGA AG-3'; GAPDH (size of PCR product: 735 bp): sense 5'-ATT CCA TGG CAG CGT CAA GG-3', antisense 5'-GTC GTT GAC GAG AAT GCC AG-3'. The first cDNA synthesis was performed following the manufacturer’s instructions. PCR was performed after a 4 min denaturation at 94°C, and the cycling of cycles of 94, 55 and 72°C each for 40 s; the number of cycles was specific for each primer set. PCR products were electrophoresed in a 1.5% agarose gel containing ethidium bromide.

**Statistical analysis**

Each experiment was performed at least three times. Results are expressed as the means value ± standard deviation (SD). Statistical analysis was performed using Student’s t-test and statistical significance is expressed as *, P < 0.05; **, P < 0.01.

**Fig. 1.** Chemical structure of panduratin A isolated from *K.pandurata.*
Inhibition of PC3 and DU145 cell growth by panduratin A

The effect of panduratin A on cell viability was determined employing a MTT assay. As shown in Figure 2, panduratin A treatment to PrEC (Figure 2A) cells produced little to no cytotoxicity in 24 h at which time it produced significant growth inhibition in PC3 and DU145 cells. At 48 and 72 h treatment this differential cytotoxic response of panduratin A persisted. At 24 h treatment with panduratin A IC₅₀ values of 13.5 and 14 μM in PC3 (Figure 2B) and DU145 (Figure 2C) cells, respectively were obtained. For further mechanistic studies a dose of 2.5, 5, 10 and 20 μM panduratin A and a treatment time of 24 h was selected.

Induction of apoptosis by panduratin A in PC3 and DU145 cells

Annexin-V/PI staining was performed to determine early, late apoptotic and necrotic cells following panduratin A treatment to PC3 and DU145 cells. Annexin-V specially binds to phosphatidylserine and has been employed for determination of apoptotic cells. When PC3 and DU145 cells were stained with annexin-V/PI and examined under a fluorescence microscope, early and late apoptosis (annexin V-stained) cells were found to be increased in panduratin A treated cells in a dose-dependent manner. Data obtained with 10 and 20 μM concentration of panduratin A is depicted in Figure 3A. The cleavage of poly (ADP-ribose) polymerase (PARP) and acinus are regarded as hallmarks for induction of apoptotic response. The cytoplasmic levels of the native PARP (116 kDa) and its cleaved product (85 kDa) and degradation of acinus were determined by immunoblot analysis in panduratin A treated cells. As shown in Figure 3B, the native 116 kDa PARP protein was found to be cleaved into its characteristic 85 kDa fragment upon treatment with panduratin A in both cells. Also, treatment of panduratin A was found to cause degradation of acinus in both cell types (Figure 3B). Taken together, these data indicate that panduratin A-induced cell death is due to the induction of apoptosis in both cell types.

Effect of panduratin A on the expression of apoptosis-related procaspases in PC3 and DU145 cells

Caspases are responsible for many of the biochemical and morphological changes that occur during apoptosis. In next series of experiments, we determined whether treatment of PC3 and DU145 cells to panduratin A leads to activation of caspase. Immunoblot analysis depicted in Figure 4 indicates that panduratin A treatment to both cell types led to activation of initiator caspases 9 and 8 as well as procaspases 6 and 3.

Panduratin A modulates Bcl-2 family proteins in PC3 and DU145 cells

In next series of experiments, we determined the effect of panduratin A treatment to PC3 and DU145 cells on levels of proteins of the Bcl-2 family members. The immunoblot analysis data showed that the expression of Bax (proapoptosis protein) was significantly increased dose-dependently and time-dependently after treatment of panduratin A in both cells (Figure 5), whereas the expression of Bcl-2 (antiapoptosis protein) was significantly decreased in a dose-dependent and a time-dependent manner. Thus, panduratin A treatment was found to result in alteration in Bax:Bcl-2 ratio in favor of apoptosis that was ~6-fold higher (at 20 μM) in PC3 cells and ~15-fold higher (at 20 μM) in DU145 cells, compared with their respective controls (Figure 5). The levels of native Bid were also significantly decreased dose-dependently in panduratin A treated cell.

Panduratin A induces Fas death receptor-mediated apoptosis in PC3 and DU145 cells

Because panduratin A induced the cleavage of initiator caspase 8, we considered that its proapoptotic response, at least in part,
Fig. 3. Panduratin A induces apoptosis in PC3 and DU145 cells as assessed by (A) fluorescence microscopy and (B) by immunoblot analysis. (A) The figures contain the representative micrographs of PC3 and DU145 cells undergoing apoptosis induced by treatment with panduratin A as assessed by fluorescence microscopy. Cells were treated with vehicle alone or specified concentration of panduratin A for 24 h. Apoptosis and necrosis was detected by a Zeiss Axiovert 100 microscope as described in Material and methods. The samples were excited at 330–380 nm, and the image was observed and photographed under a combination of a 400 nm dichoric mirror and the 420 nm high pass filter. Data from a typical experiment repeated effects are shown; Magnification ×200. (B) Cells were treated with specified concentrations of panduratin A for 24 h, harvested and total cell lysates were prepared. PARP cleavage and the expression of acinus were determined by western blot analysis. Equal loading was confirmed by stripping immunoblots and reprobing them for β-actin. A typical immunoblot from three separate experiments with similar results is shown here. The details are described under Materials and methods.

Fig. 4. Dose-dependent effect of panduratin A on the expression of apoptosis-related procaspases in PC3 and DU145 cells. Cells were treated with specified concentrations of panduratin A for 24 h, harvested and total cell lysates were prepared. The expression of procaspases 3, 6, 8 and 9 were determined by western blot analysis. Equal loading was confirmed by stripping immunoblots and reprobing them for β-actin. A typical immunoblot from three separate experiments with similar results is shown here. The details are described under Materials and methods.
may be mediated via the death receptor-signaling pathway. As shown in Figure 6, in panduratin A treated PC3 and DU145 cells, a significant increase in the expression of fas-associated death domain (FADD) protein was observed. Panduratin A treatment was found to cause a dose-dependent upregulation of the Fas receptor protein without a significant change in the expression of Fas-L in both cells. In addition, a significant change in the expression of TNF-related apoptosis-inducing ligand (TRAIL) was also observed in both cell lines (Figure 6). These data suggest that panduratin A treatment also induces apoptosis through extrinsic pathway involving the death receptors.

**Panduratin A treatment caused G2/M phase arrest in PC3 and DU145 cells**

To investigate whether panduratin A treatment has an effect on the cell cycle regulation, we determined its effect on cell cycle distribution by flow cytometry after staining with PI. As shown in Figure 7, concomitant with growth inhibitory effects, panduratin A treatment induced a strong G2/M phase arrest in PC3 and DU145 cells.
a dose-dependent manner. In PC3 cells G2/M phase distribution was 12.4, 14.9, 21.0 and 38.6% at 2.5, 5, 10 and 20 μM concentrations of panduratin A, respectively (Figure 7A). In DU145 cells G2/M phase distribution was 17.0, 25.9, 22.0 and 38.6% at 2.5, 5, 10 and 20 μM concentrations of panduratin A, respectively (Figure 7B). Interestingly, the increase in cell population in G1 phase was observed at a concentration of 2.5 μM panduratin A treatment in both the cells.

**Effect of panduratin A on the expression of cell cycle regulators in PC3 and DU145 cells**

Since panduratin A was observed to cause an arrest of cells in G2/M phase, we next assessed its effect on G2/M cell cycle regulators including cdc25C, p-cdc2, cdc2 and cyclin B1. As shown by immunoblot analysis in Figure 8A, panduratin A treatment of cells caused a dose-dependent decrease in the levels of cdc25C, cdc2 and cyclin B1 proteins. However, the phosphorylation of cdc2 was found to be decreased only
Fig. 8. Effect of panduratin A treatment to PC3 and DU145 cells on change in G2/M cell cycle regulator, other cell cycle regulator and cyclin-dependent kinase inhibitor (cdkI). Cells were treated with specified concentrations of panduratin A for 24 h, harvested and total cell lysates were prepared. The expression of (A) Cyclin B1, cdc2, p-cdc2 and cdc25C; (B) cyclin D1, cdk6, cdk4, cyclin E and cdk2 and (C) cdkI (p27 Kip1 and p21 WAF1/Cip1) were determined by western blot analysis. Equal loading was confirmed by stripping immunoblots and reprobing them for β-actin. A typical immunoblot from three separate experiments with similar results is shown here. The details are described under Materials and methods.
in PC3 and not in DU145 cells treated with panduratin A (Figure 8A). Next, we assessed the effect of panduratin A on the expression of cyclins D1, E and cyclin dependent kinases (cdks) 2, 4 and 6. As shown in Figure 8B, the expression level of cyclins D1 and E1 significantly decreased following panduratin A treatment in both cell types. We also observed a decrease in the expression of cdk5 by panduratin A treatment in both cell lines.

Effect of panduratin A on p27Kip1 and p21Waf1/Cip1 in PC3 and DU145 cells

Association of cyclin with cdk forms active kinase complexes, which are regulated and inhibited by binding with cdki. We next determined the effect of panduratin A treatment of cells on the cytoplasmic levels of p27Kip1 and p21Waf1/Cip1. As shown by immunoblot analysis in Figure 8C, the expression level of p27Kip1 was increased in a dose-dependent manner by panduratin A treatment in both cell lines. We also determined the effect of panduratin A treatment of cells. Similar to p27Kip1 protein expression, RT–PCR analysis indicated that expressed p27Kip1 mRNA was increased in a time-dependent manner by panduratin A treatment in both cell lines (Figure 9). These data show the possibility for the involvement of p27Kip1 in panduratin A-induced G1 or G2/M phase arrest in both cell lines.

Discussion

CaP is a common malignancy and is the second leading cause of cancer-related deaths of males in the USA with similar trend in many western countries. Because CaP usually occurs in men aged 50 years and older and because of increasing life expectancy, its incidence is expected to continue to rise in the years to come (12). CaP usually progresses from androgen-dependent to androgen-independent stage, making antiandrogen therapy ineffective leading to an increase in metastatic potential and incurable malignancy (1,2). Recently, chemoprevention and intervention strategies using anticancer agents are suggested as promising alternative options. The search for new chemopreventive and/or chemotherapeutic agents that are more effective without toxic effects has generated great interest in identifying phytochemicals for their potential use for humans (3,4,13,14).

Suppression of tumorigenesis often involves modulation of signal transduction pathways, leading to alterations in gene expression, arrest in cell cycle progression or apoptosis. Apoptosis represents a universal and exquisitely efficient suicidal pathway, considered as an ideal way for elimination of damaged cells (15). Recently, the apoptosis signaling systems have been shown to provide promising targets for the development of novel anticancer agents (16). Several plant-derived bioactive agents are known to be chemopreventive agents inducing apoptosis in a number of experimental models of carcinogenesis (17–19). Thus induction of apoptosis is considered as a possible mechanism of chemopreventive agents.

Panduratin A (Figure 1) is a cyclohexenyl chalcone derivative isolated from K. pandurata. Chalcones are natural products, which have been reported to possess a variety of biological effects, including anti-inflammatory, analgesic and anti-oxidant properties (7). Recently we have shown that panduratin A possesses anti-inflammatory effects in RAW264.7 cells (8). However, little is known about the effect of panduratin A on experimental carcinogenesis. As a step towards evaluating an anti-tumorigenic potential of this compound, we have recently shown that it induces apoptosis in HT-29 cells (9). Interestingly, we found that in colon cancer cells panduratin A was a more potent inhibitor of growth than selective COX-2 inhibitor Celecoxib and antitumor drugs (5-fluorouracil and cisplatin). This prompted us to explore the effect of panduratin A on human CaP cells. In the present study, we investigated the anti-proliferative activity of panduratin A and the underlying mechanism in androgen-independent human prostate cancer PC3 and DU145 cells.

Caspases plays a central role in many forms of apoptosis (20,21). These enzymes are synthesized as inactive zymogens that must be cleaved after conserved aspartate residues to be activated. Both the intrinsic and extrinsic pathways have been shown to trigger caspase activation in cells undergoing apoptosis (22,23). In the mitochondrial pathway (intrinsic pathway), the death signals are relayed to mitochondria where release of cytochrome c is induced. Cytochrome c binds to Apaf-1, participating in formation of the apoptosome-dATP-dependent complex, which activates caspase 9. Active caspase 9 can then cleave downstream effector caspases (24–26). The extrinsic pathway for cell death involves plasma membrane death receptors (3,22). These receptors trimerize and recruit the adaptor molecule FADD, which, in turn, activates caspase 8. This, in turn, also leads to the activation of downstream execution caspases (27–30). In both pathways, activation of effector caspases leads to a series of morphological changes characteristics of apoptosis (15).
In this study, panduratin A treatment to CaP cells led to a remarkable induction of apoptotic cells. Panduratin A treatment also led to activation of initiator caspase 8 and 9 as well as that of downstream effector caspases 3 and 6. The activation of effector caspases 3 and 6 in response to panduratin A treatment also resulted in cleavage of PARP and acinus in both cell types. The ratio of Bax:Bcl-2 is critical to cell survival such that an increase in Bax levels can shift the process in favor of apoptosis (31). Panduratin A-induced apoptosis and cell growth inhibition was accompanied with decrease in Bcl-2 with concomitant increase in Bax in both cell types. This altered expression of Bcl-2 family members by panduratin A treatment may trigger the activation of initiator caspases 8 and 9 followed by activation of effector caspases 3 and 6. This, in turn, also may lead to cleavage of PARP and acinus.

The Fas receptor (CD95 or Apo1) is the most completely characterized death receptor. Studies have demonstrated that apoptosis induced by anticancer therapy involves the CD95 system (32). TRAIL is a member of the TNF family and can also induce apoptotic cell death in a variety of cell types including CaP cells (32,33). Stimulation of death receptors of the TNF receptor superfamily such as CD95 (APO-1/Fas) or TRAIL receptors results in receptor aggregation and recruitment of the adaptor molecule FADD (28,34,35). Upon recruitment, caspase 8, becomes activated and initiates apoptosis by direct cleavage of downstream effector caspases 3 and 6. Activation of caspase 8 then leads to a series of downstream apoptotic events (29,30). Studies have shown that the death receptor-induced apoptosis is linked to the mitochondrial machinery through Bid (36,37). Furthermore, the death signal form the Fas receptor may be linked to mitochondria by Bid, a Bcl-2 family containing protein of the Bcl-2 family (38). Bid then translocates to the mitochondria and mediates the release of cytochrome c that leads to apoptotic changes (38). In this study, panduratin A induced expression of Fas and TRAIL in PC3 and DU145 cells which may, in turn, lead to aggregation and recruitment of the adapter protein FADD, which results in the activation of caspase 8. These sequence of events may also leads to the activation of downstream execution caspases. Several lines of evidence suggest that both TRAIL and FAS signaling pathways are involved in chemotherapy-induced apoptosis, either by activating the initiator caspase 8 or at the level of downstream effector caspase (caspases 3, 6 and 7) activation (39,40). Thus, it is likely that panduratin A-induced apoptosis of CaP cells follows this pathway.

Induction of apoptosis and/or inhibition of cell proliferation are highly correlated with the activation of a variety of intracellular signaling pathways leading to arrest the cell cycle in the G1, S, or G2/M phase of the cell cycle (15) Cell cycle regulation and its modulation by various plant-derived agents are gaining widespread attention in recent years. A large number of phytochemicals have been shown to inhibit cell cycle progression of various cancer cells (41). Especially, G2/M transition provides an effective checkpoint in cell cycle progression that is regulated by the sequential activation and deactivation of cdc family proteins and cyclin complexes. cdc2 interacts with cyclin B1, and activation of the cyclin B–cdc2 complex is required for transition from G2 to M phase of the cell cycle (42–44). Moreover, cdc25C functions as a mitotic activator by dephosphorylating cdc2 that forms a complex with cyclin B1 and drives the cell from G2→M phase. The activation of cdc25C is controlled via its inhibitory phosphorylation by upstream kinase Chk1/2, in response to DNA damage, and thus inhibits mitosis (45–49). In the present study, we found that panduratin A treatment resulted in G2/M phase arrest in a dose-dependent manner in both PC3 and DU145 cells. We further investigated the effect of panduratin A on the expression of G2/M regulatory proteins. Panduratin A treatment was found to result in a remarkable decrease in the protein levels of cyclin B1, cdc25C and cdc2 in both cell types. These results suggest that reduced expression of cyclin B1 and cdc2 and cdc25C may be involved in panduratin A-induced G2/M phase arrest, leading to cell growth inhibition and possible apoptotic death. The effect of panduratin A on the expression of other cyclins and their corresponding protein partners were also investigated. We found that treatment of cells to panduratin A resulted in a dose-dependent decrease in the protein expression of cyclins D1 and E1 with a concomitant decrease in both cell types. These data indicate that panduratin A modulates multiple regulatory molecules important in the cell cycle regulation.

Studies have demonstrated that cell cycle arrest at G2/M transition by DNA damaging agent is also tightly associated with the induction of p21WAF/Cip1 (50–53). The p27Kip1 is another member of cdkIs, which could bind and inhibit a broader range of cdk5 (54–57). We found that p27Kip1 and/or p21WAF/Cip1 were upregulated in PC3 and DU145 cells treated with panduratin A. Therefore, the upregulation of p21WAF/Cip1 and/or p27Kip1 by panduratin A is, at least in part, probably responsible for the downregulation of cyclins and cdk5 expression. Moreover, the upregulation of p21WAF/Cip1 and p27Kip1 and the downregulation of cdk5 may be one of the molecular mechanisms by which panduratin A inhibits CaP cell growth and induces cell-cycle arrest.

Taken together, this study provided first evidence that panduratin A inhibited cell proliferation of CaP cells by apoptosis which was associated with upregulation of the Fas death receptor and G2/M phase arrest resulting in upregulation of the cdk inhibitor p21WAF/Cip1 and p27Kip1 and inhibition of cdk5, cyclins and cdc2/cdc25C. These abilities of panduratin A to induce apoptosis and arrest of cell cycle implies its potential as chemotherapeutic agent because many anticancer drugs are known to achieve their antitumor function by inducing apoptosis and cell cycle arrest in tumor cells. Although the precise molecular mechanism by which apoptosis is induced by panduratin A remains unclear, it might be a potent useful antitumor agent against CaP.

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