Journal of Marine Bioscience and Biotechnology **2006**, p. 243-251 Vol. 1, No. 4

Review

Molecular Mechanisms of Cell Cycle Arrest and Apoptosis by Dideoxypetrosynol A, a Polyacetylene from the Sponge *Petrosia* sp., in Human Monocytic Leukemia Cells

Yung Hyun Choi

Department of Biochemistry, Dongeui University College of Oriental Medicine and Department of Biomaterial Control (BK21 program), Dongeui University Graduate School, Busan 614-052, Korea

Abstract Dideoxypetrosynol A, a polyacetylene from the marine sponge *Petrosia* sp., is known to exhibit significant selective cytotoxic activity against a small panel of human tumor cell lines, however, the mechanisms of which are poorly understood. In the present study, it was investigated the further possible mechanisms by which dideoxytetrosynol A exerts its anti-proliferative action in cultured human leukemia cell line U937. We observed that the proliferation-inhibitory effect of dideoxypetrosynol A was due to the induction of G1 arrest of the cell cycle and apoptosis, which effects were associated with up-regulation of cyclin D1 and down-regulation of cyclin E without any change in cyclin-dependent-kinases (Cdks) expression. Dideoxypetrosynol A markedly induced the levels of Cdk inhibitor p16/INK4a expression. Furthermore, down-regulation of phosphorylation of retinoblastoma protein (pRB) by this compound was associated with enhanced binding of pRB and the transcription factor E2F-1. The increase in apoptosis was associated with a dose-dependent up-regulation in pro-apoptotic Bax expression and activation of caspase-3 and caspase-9. Dideoxytetrosynol A decreased the levels of cyclooxygenase (COX)-2 mRNA and protein expression without significant changes in the levels of COX-1, which was correlated with a decrease in prostaglandin E_2 (PGE_2) synthesis. Furthermore, dideoxytetrosynol A treatment markedly inhibited the activity of telomerase, and the expression of human telomerase reverse transcriptase (hTERT), a main determinant of the telomerase enzymatic activity, was progressively down-regulated by dideoxytetrosynol A treatment in a dose-dependent fashion. Taken together, these findings provide important new insights into the possible molecular mechanisms of the anti-cancer activity of dideoxytetrosynol A.

Key words : Dideoxypetrosynol A, sponge *Petrosia* sp., U937, G1 arrest, pRB, p16/INK4a, apoptosis, cyclooxygenase, telomerase

Introduction

Recent studies indicated that the marine environment has proven to be a very rich source of bioactive compounds [23,28]. Marine sponges, a kind of sedentary marine species, have been used as a main source for this study of hitherto unknown biological activities of natural marine products. Components of marine sponges are known to modulate various biological activities such as anti-tumor, anti-inflammatory or immunomodulatory effects [17,24,30]. Through screening of natural compounds that induce cell cycle arrest and/or apoptosis, we previously reported dideoxypetrosynol A, a polyacetylene from the sponge *Petrosia* sp., has significant selective cytotoxic activity against a small panel of human solid tumor cell lines by inhibiting DNA replication and apoptotic cell death [6,17,19], however, the molecular mechanisms of its anti-proliferative action on malignant cell growth are not known completely. The present study was carried out to characterize the probable mechanisms involved in dideoxype-

* Corresponding author
Phone: +82-51-857413, Fax: +82-51-853-4036
E-mail: choiyh@deu.ac.kr

trosynol A-mediated growth inhibitory effect in human monocytic leukamia U937 cells. We demonstrated that dideoxypetrosynol A induced cell cycle arrest at G1 phase and apoptosis through a combined mechanism involving the induction of Cdk inhibitor p16 and the down-regulation of phosphorylation of pRB. Our study also revealed that dideoxypetrosynol A exhibited a significant inhibition on the growth of U937 cells in parallel with the reduction of COX-2 and hTERT which was associated with an inhibition of PGE_2 release and telomerase activity. These results suggested that dideoxypetrosynol A is a potential agent for cancer treatment.

G1 arrest and induction of apoptosis by dideoxytetrosynol A by dideoxypetrosynol A

To evaluate the effects of dideoxypetrosynol A (Fig. 1A) on cell proliferation, we initially determined the effects of dideoxypetrosynol A on the growth of U937 cells. For this purpose, cells were treated with dideoxypetrosynol A (0.2-1.0 μg/ml) and were measured by MTT assay. As shown in Fig. 1B, dideoxypetrosynol A had a strong inhibitory effect on cell proliferation in a dose-dependent manner, which was associated with a distinct morphological change including membrane ruffling (data not shown). In order to determine whether the growth inhibition by dideoxytetrosynol A was associated with the induction of apoptosis, DAPI staining was performed. As shown in Fig. 1C, the control cells displayed intact nuclear structure, while nuclei with chromatin condensation and formation of apoptotic bodies, a characteristic of apoptosis, were seen in cells cultured with dideoxytetrosynol A in a dose-dependent fashion. We also analyzed whether DNA fragmentation, another hallmark of apoptosis, was induced by dideoxytetrosynol A in U937 cells. Following agarose gel electrophoresis of U937 cells treated with 0.5 μg/ml and 1.0 μg/ml dideoxytetrosynol A for 48 h, a typical ladder pattern of internucleosomal fragmentation was observed (Fig. 1D). We next analyzed the amount of sub-G1 DNA, which contained less DNA than G1 cells, to quantify the degree of dead cells. Flow cytometric analysis indicated that dideoxytetrosynol A treatment resulted in a markedly increased accumulation of sub-G1 phase in a dose-dependent manner (Fig. 1E). Taken together, these results demonstrated that the cy-

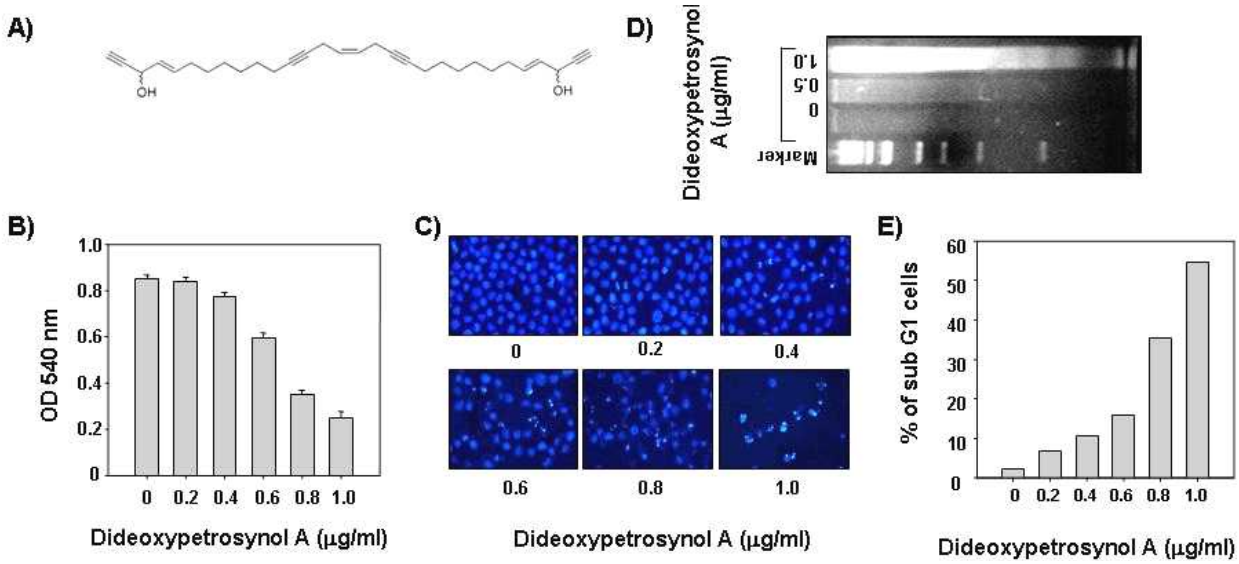

Fig. 1. Growth inhibition and apoptosis by dideoxypetrosynol A treatment in U937 cells. (A) Chemical structure of dideoxypetrosynol A. (B) Cells were plated at 4 x 10^4 cells per 60-mm plate, and incubated for 24 h. The cells were treated with variable concentrations of dideoxypetrosynol A for 48 h and growth inhibition was measured by the metabolic-dye-based MTT assay. Results are expressed as the means ± S.E. of three independent experiments. (C) Cells were cultured for 48 h with vehicle alone or dideoxytetrosynol A before cells were fixed and stained with DAPI. After 10 min incubation at room temperature, stained nuclei were then observed under a fluorescent microscope using a blue filter. Magnification, X400. (D) To analyze fragmentation of genomic DNA, cells were treated for 48 h with the indicated concentrations of dideoxytetrosynol A. Fragmented DNA was extracted and analyzed on 1% agarose gel. (E) Cells treated for 48 h with increasing concentration of dideoxytetrosynol A. Then the cells were collected and stained with PI for flow cytometry analysis. The fraction of apoptotic sub-G1 cells is indicated. Data are presented as the mean values obtained from two independent experiments.

totoxic effects observed in response to dideoxytetrosynol A is associated with the induction of apoptotic cell death. To determine whether dideoxypetrosynol A treatment of cells resulted in the alteration of cell cycle progression, the cell cycle patterns of the U937 cel1s were examined. Analysis of the cell cycle distribution of cells after exposure to the dideoxypetrosynol A showed that these cells had marked accumulation in G1 phase of the cell cycle (Table 1). This was accompanied by a decrease in their S and G2/M phase when compared with the untreated control cells, which suggests that the growth inhibitory effect of dideoxypetrosynol A in U937 cells was the result of a block during this G1 phase.

Effects of dideoxypetrosynol A on the levels of cell cycle regulators

Since dideoxypetrosynol A arrested U937 cells in the G1 phase of the cell cycle, we determined the expression levels of the cell cycle regulating factors at the G1 boundary, such as cyclin D1, cyclin E, Cdk2, Cdk4 and Cdk6, by RT-PCR and Western blotting. As shown in Fig. 2A & B, the protein and mRNA levels of cyclin D1 were significantly increased by dideoxypetrosynol A treatment and the levels of cyclin E were decreased in a concention-dependent manner. However, the levels of Cdks (Cdk2, Cdk4 and Cdk6) remained unchanged in dideoxypetrosynol A-treated cells. These results suggest that the suppressive effects of the dideoxypetrosynol A at G1 phase of U937 cells are partly caused by up-regulating the levels of cyclin D1 and down-regulating the levels of cyclin E. To further understand the anti-proliferative mechanism of dideoxypetrosynol A, we next investigated whether Cdk inhibitors, such as p16/INK4a, p21/WAF1/CIP1 and p27/KIP1, are involved in the dideoxypetrosynol A-induced growth arrest in U937 cells (Fig. 2A & B). In the untreated control cells, the protein and mRNA levels of p16 were undetectable. However, the incubation of cells with dideoxypetrosynol A caused a striking concentration-dependent increase in the induction of p16 protein and mRNA, whereas the compound did not significantly affect the expression levels of other Cdk inhibitors, including p21 and p27. The data suggested that the dideoxypetrosynol A-induced G1 cell cycle arrest in U937 cells requires increased p16 expression, but not p21 and p27 expression. Since the *RB* gene product pRB is an important checkpoint protein in G1 phase of the cell cycle, we next determined the kinetics between phosphorylation of pRB and the transcription factor E2F-1. The total levels of pRB expressions were

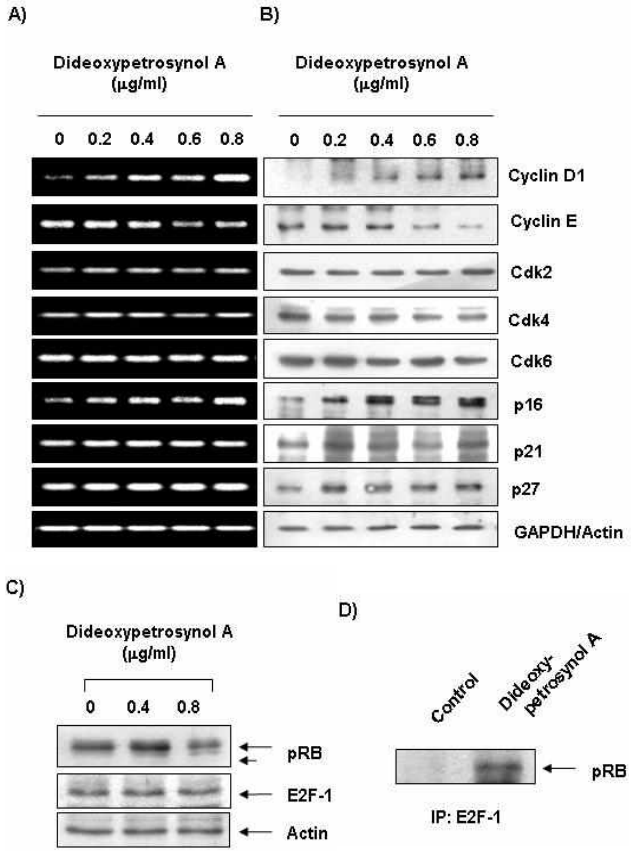

Fig. 2. Effects of dideoxypetrosynol A on the protein levels of several cell cycle regulators U937 cells. (A) After 48 h incubation with dideoxypetrosynol A, total RNAs were isolated and reverse-transcribed. The resulting cDNAs were subjected to PCR with indicated primers and the reaction products were subjected to electrophoresis in a 1% agarose gel and visualized by EtBr staining. GAPDH was used as an internal control. (B) The cells were lysed and then cellular proteins were separated by SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. The membranes were probed with the indicated antibodies. Proteins were visualized using an ECL detection system. Actin was used as an internal control. (C) Cells were treated with the indicated concentrations of dideoxypetrosynol A for 48 h and total cell lysates were prepared and separated by 8% or 10% SDS-polyacrylamide gel. Western blotting was performed using anti-pRB and anti-E2F-1 antibodies. (D) Whole cell lysates (0.5 mg of protein) from control cells and cells treated with dideoxypetrosynol A were immunoprecipitated with anti-E2F-1 antibody. Immuno-complexes were separated by 8% SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose membrane, and probed with anti-pRB antibody. Proteins were detected by ECL detection.

Table 1. Effect of dideoxypetrosynol A on the distribution of the cell cycle in U937 cells. Cells have been grown in different concentrations of dideoxypetrosynol A for 48 h, and then analyzed by flow cytometry as described in Materials and Methods. Data are presented as the mean values obtained from two independent experiments

dideoxypetrosynol A (μg/ml)	% of cell		
	G1	S	G2/M
0	46.29	28.74	24.97
0.2	53.89	25.07	21.04
0.4	57.07	23.02	19.91
0.6	60.37	22.55	17.08
0.8	63.84	20.24	15.92

decreased remarkably, and changed from hyperphosphorylated form to hypophosphorylated form by dideoxypetrosynol A treatment without altering E2F-1 expression (Fig. 2C). Co-immunoprecipitation analysis indicated that association of pRB and E2F-1 was almost undetectable in the untreated log phase cells, however, there was a strong increase in the association of pRB and E2F-1 in dideoxypetrosynol A-treated cells (Fig. 2D) suggesting that dideoxypetrosynol A inhibits the releasing of E2F-1 protein from pRB.

Induction of Bax protein and activation of caspases by dideoxytetrosynol A

In light of the importance of the expression of the Bcl-2 protein family on the regulation of apoptosis, we evaluated the levels of Bax, Bcl-2 and Bcl-xL in dideoxytetrosynol A-treated U937 cells. Western blot analysis revealed that the anti-apoptotic Bcl-2 and Bcl-xL levels remained virtually unchanged in response to dideoxytetrosynol A (Fig. 3), whereas pro-apoptotic Bax expression was increased by dideoxytetrosynol A treatment, suggesting dideoxytetrosynol A alters the Bax:Bcl-2 and Bax:Bcl-xL ratio in U937 cells. To gain further insight into the mechanism by which dideoxytetrosynol A induces apoptosis, we examined the effects of dideoxytetrosynol A on caspase-3 and caspase-9 protein levels and their activity. As shown in Fig. 4A, dideoxytetrosynol A-induced apoptosis of U937 cells was associated with a decreased expression of the pro-caspase-3 and pro-caspase-9 protein; however, the active subunits of both caspases were not observed. To quantify the activities of caspase-3 and caspase-9, lysates equalized for protein from cells treated with dideoxytetrosynol A were assayed for *in vitro* caspase-3 and caspase-9 activity using DEVD-pNA and LEHD-pNA, respectively, as fluorogenic substrates. As indicated in Fig. 3B, the results showed that dideoxytetrosynol A exposure markedly increased both caspase-3 and caspas-9 activity in a concentration-dependent manner. Furthermore, dideoxytetrosynol A induced a concomitant degradation of PARP protein and accumulation of the 85 kDa, which is a substrate protein of caspase-3. We also examined the effects of dideoxytetrosynol A on the anti-apoptotic inhibitory apoptosis proteins (IAPs) family, which bind to caspases and lead to inactivation of caspases. As shown in Fig. 3A, dideoxytetrosynol A markedly inhibited cIAP-1 and cIAP-2, but not XIAP in U937 cells.

Inhibition of COX-2 activity and PGE2 production by dideoxytetrosynol A

We next determined whether dideoxytetrosynol A-induced anti-proliferative effect of U937 cells was connected with a reduced activity of COXs. The RT-PCR and Western blot analyses showed a significant decrease

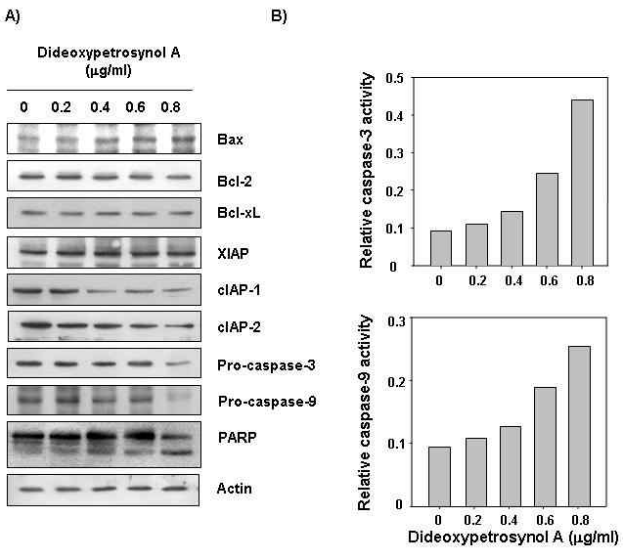

Fig. 3. Effects of dideoxytetrosynol A on the levels of apoptotsis regulators expression in U937 cells. (A) After 48 h incubation with dideoxytetrosynol A, cells were lysed and then cellular proteins were separated by 10% SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. The membranes were probed with the indicated antibodies. Proteins were visualized using an ECL detection system. Actin was used as an internal control. (B) Cell lysates from cells treated with dideoxytetrosynol A for 48 h were assayed for in vitro caspase-3 and -9 activity using DEVD-pNA and LEHD-pNA-pNA, respectively, as substrates at 37°C for 1 h. The released fluorescent products were measured. Data are presented as the mean values obtained from two independent experiments.

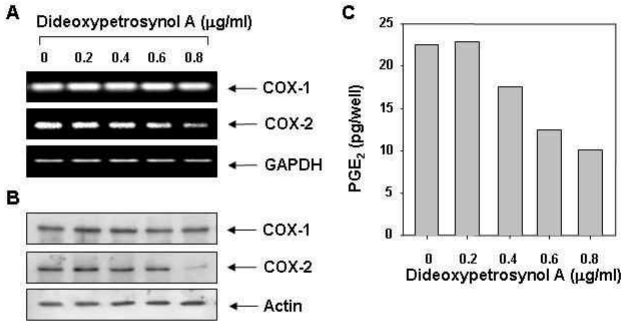

Fig. 4. Down-regulation of COX-2 expression and inhibition of PGE_2 production by dideoxytetrosynol A in U937 cells. (A) After 48 h incubation with dideoxytetrosynol A, total RNAs were isolated and reverse-transcribed. The resulting cDNAs were subjected to PCR with COX-1 and COX-2 primers and the reaction products were subjected to electrophoresis in a 1% agarose gel and visualized by EtBr staining. GAPDH was used as an internal control. (B) Cells were treated with the indicated concentrations of dideoxytetrosynol A for 48 h and collected. The cells were lysed and then cellular proteins were separated by 10% SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. The membranes were probed with the antibodies against COX-1 and COX-2. Proteins were visualized using an ECL detection system. Actin was used as an internal control. (C) Cells were treated with the indicated concentrations of dideoxytetrosynol A for 48 h and collected. The PGE_2 accumulation in the medium was determined by an EIA kit as described in materials and methods. Data represent the relative mean values of two independent experiments.

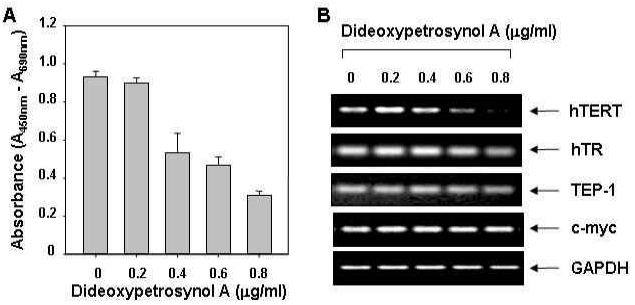

Fig. 5. Inhibition of telomerase activity by dideoxytetrosynol A treatment in U937 cells. (A) After 48 h of incubation with dideoxytetrosynol A, telomerase activity of U937 cells were measured using a TRAP-ELISA kit as described in materials and methods. For one sample, 2×10^5 cells were lysed, and 1/100 was used in the assay. Data represents the relative mean values ± S.E. of three independent experiments. (B) After 48 h of incubation with dideoxytetrosynol A, total RNA was isolated and RT-PCR was performed using indicated primers. The amplified PCR products were run in a 1% agarose gel and visualized by EtBr staining. GAPDH was used as a house-keeping control gene.

in COX-2 mRNA and protein expression after dideoxytetrosynol A treatment but, dideoxytetrosynol A was ineffective on COX-1 expression (Fig. 4A and B). To confirm that PGE_2 production was associated with the catalytic activity of COX-2 isoform, U937 cells were cultured in the presence of dideoxytetrosynol A, and PGE_2 levels were measured. As shown in Fig. 5C, the synthesis of PGE_2 was concentration-dependent and this production was significantly decreased over time after dideoxytetrosynol A treatment, which was well correlated with down-regulation of COX-2 expression.

Inhibition of telomerase activity and hTER expression by dideoxytetrosynol A

To examine the effects of dideoxytetrosynol A on telomerase activity, U937 cells were cultured with various concentrations of dideoxytetrosynol A for 48 h and telomerase activity was measured by TRAP-ELISA. As shown in Fig. 6A, telomerase activity was reduced by dideoxytetrosynol A treatment in a dose-dependent manner. Of the components comprising telomerase, hTERT is a critical determinant of the enzyme activity of telomerase. Therefore we examined changes in hTERT expression on treatment with dideoxytetrosynol A using the RT-PCR assay. As indicated in Fig. 6B, hTERT mRNA expression, but not hTR, TEP-1 and c-myc, was markedly decreased by treatment with dideoxytetrosynol A in a concentration-dependent fashion suggesting that the repression of telomerase activity by dideoxytetrosynol A was associated with down-regulation of hTERT mRNA.

Discussion

In this study, we tested dideoxypetrosynol A, a polyacetylene from the marine sponge *Petrosia* sp., for its activity in inhibiting the growth of human monocytic leukamia U937 cells. We found that treatment of cells with dideoxypetrosynol A resulted in a concentration-dependent inhibition of cell viability, which was associated with gross morphological changes. Subsequent experiments addressed the issue of whether this compound perturbs the cell cycle using DNA flow cytometric analysis. The data revealed a cell-cycle block at G1 to S phase transition and an accumulation of cells at sub-G1 apoptotic region (data not shown), which contained less DNA than G1 cells [21]. These results

suggested that dideoxypetrosynol A interferes with the proliferation of U937 cells and induces apoptosis in close association with the G1 arrest by modulation of cell cycle-regulators expression as a possible molecular mechanism of the effect of dideoxypetrosynol A. Thus, we investigated the effects of dideoxypetrosynol A on the expression of G1/S transition regulatory proteins to analyze the mechanism of G1 arrest.

In terms of regulation of the cell cycle, Cdks play a most critical role. Two major mechanisms for Cdk regulation are binding with its catalytic subunit cyclin, followed by activation of Cdk/cyclin complexes, and binding with Cdk inhibitors followed by inactivation of Cdk/cyclin complexes [35,40]. An alteration in the formation of these complexes could lead to increased cell growth and proliferation, and decreased cell growth and proliferation followed by differentiation and/or cell death by apoptosis [13]. In general, cyclin D1 is synthesized in a pre-DNA-synthetic gap (early G1 phase) and is the key regulator of the signal transduction in the G1 phase cell proliferation. If cyclin D1 is overexpressed, the checkpoint of G1/S will be out of control and lose its role in the signaling of proliferation [22, 26]. However, other recent results show that when cell cycle blockage occurs in the G1 phase, cyclin D1 is not decreased, but accumulated in cells, suggesting that G1 arrest does not always follow the decrease of cyclin D1 expression. And, the progression through the G1/S transition is regulated by cyclin E, which is expressed in late G1 preceding cyclin A expression, with maximal expression at the G1/S boundary [18,27]. In the present study, the results from RT-PCR and immunoblotting analysis clearly demonstrated that levels of cyclin D1 expression were markedly induced by dideoxypetrosynol A treatment in both transcriptional and translational levels, but the levels of cyclin E were concentration-dependently inhibited in dideoxypetrosynol A-treated U937 cells without changes of the expression of Cdks (Fig. 2).

On the other hand, in conjunction with Cdk4/6, cyclin D mediates the initial phosphorylation of pRB. The kinase activities of cyclin E/Cdk2 and cyclin A/Cdk2 complexes then act to maintain pRB in the hyper-phosphorylated state [27]. Any factor affecting the activity of these kinases could abrogate the normal inactivation of pRB and cause an accumulation of cells in G1. Normally, pRB binds to the members of the E2F family of transcription factors. In response to the growth factors, the pRB is phosphorylated and dissociated from E2F, which triggers G1 cell cycle progression [9,16]. Thus, an obvious candidate for control of pRB phosphorylation is the cyclin E/Cdk2 complex. If decreased levels of either protein or association between respective binding partners were observed, a concomitant decrease in the degree of pRB phosphorylation would be expected [16]. pRB is also known to inhibit the transcriptional activation of p16 expression in the cells, and the genetic mutation of the *RB* gene resulted in high levels of p16 expression [20,33]. Thus, p16 has the capacity to arrest cells in the G1-phase of the cell cycle and its probable physiological role is in the implementation of irreversible growth arrest, termed cellular senescence. Our data showed that treatment with dideoxypetrosynol A in U937 cells inhibited the phosphorylation of pRB and enhanced the association of pRB and E2F-1 (Fig. 2). Furthermore, we found that the treatment of cells with dideoxypetrosynol A selectively induced p16 expression in a dose-dependent manner indicated that neither p21 nor p27 was involved in the G1 arrest which was induced by dideoxypetrosynol A in U937 cells.

The regulation of apoptosis is a complex process and involves a number of gene products including Bcl-2 protein family. It has been reported that Bcl-2 protects against multiple signals that lead to cell death, indicating that Bcl-2 regulates a common cell death pathway and functions at a point where various signals converge [25,31]. Bcl-2 acts to inhibit cytochrome *c* translocation from mitochodria to cytoplasm, thereby blocking the caspase activation step of the apoptotic process [32,31]. Thus, it has been suggested that the ratio between the level of pro-apoptotic Bax protein and that of the anti-apoptotic factor Bcl-2 protein determines whether a cell responds to an apoptotic signal [34]. In our study, there was a concentration-dependent increase of Bax protein levels in U937 cells treated with dideoxytetrosynol A, but the levels of Bcl-2 members such as Bcl-2 and Bcl-xL remained unchanged, resulting in an increase in the ratio of Bax/Bcl-2 and Bcl-xL (Fig. 3). The caspase family also plays an important role in driving apoptosis and the key components of the biochemical pathways of caspase activation have been recently elucidated [12]. They are synthesized initially as single polypeptide chains representing latent precursors that undergo proteolytic processing at specific residues to produce subunits that form the active hetero-

tetrameric protease. IAPs family proteins reportedly block apoptosis due to their function as direct inhibitors of activated effector caspases (caspase-3 and caspase-7). Furthermore, cIAP1 and cIAP2 inhibit cytochrome *c*-induced activation of caspase-9 [11,32]. Further studies have shown that exposure of U937 cells to dideoxytetrosynol A caused a proteolytic activation of caspase-3 and caspase-9 (Fig. 3), and a down-regulation of cIAP-1 and cIAP-2 (Fig. 3). Activated caspases induce a limited proteolysis in a number of cellular proteins, which are degraded as a consequence of apoptosis by the caspase family and have been used as a marker of chemotherapy-induced apoptosis [36, 39]. Here, we examined whether PARP protein, a substrate of caspase-3 [38], was cleaved in cells treated with dideoxytetrosynol A. As expected, PARP protein was clearly degraded in a dose-dependent manner, again correlating with an activation of capases during apoptosis by dideoxytetrosynol A treatment. Taken together, our data indicated that the apoptotic effects of dideoxytetrosynol A on U937 cells are associated with an up-regulating the pro-apoptotic Bax, activation of caspases and the alteration of IAPs expression.

The conversion of arachidonic acid to PGs is catalyzed by two isoenzymes, COX-1 and COX-2. COX-1 is expressed in most tissues that generate PGs during their normal physiological functions, and its expression does not fluctuate in response to stimuli [8]. However, COX-2 expression can be induced by various agents, including inflammatory cytokines, mitogens, reactive oxygen intermediates and many other tumor promoters. In a number of experimental studies, induction of COX-2 has been shown to promote cell growth, inhibit apoptosis and enhance cell motility [10,14]. It has also been reported that the overexpression of COX-2 in normal cells leads to increased carcinogenesis, metastatic potential and angiogenesis and increased expression of COX-2 has been reported in many cancer [4]. Although the exclusive role of COX-2 in tumor development and progression has yet not been fully elucidated, the development of specific COX-2 inhibitors promise to be an effective approach in the prevention and treatment of cancer [10,29]. In the present study, we observed that dideoxytetrosynol A markedly inhibited COX-2 mRNA and protein expression in U937 cells, however, the levels of COX-1 remained unaltered (Fig. 4A and B). Additionally, the amount of PGE_2 produced by U937 cells was decreased with the increase of dideoxytetrosynol A concentration (Fig. 4C). The data suggested that the inhibition of the COX-2 expression and PGE_2 production is consistent with the results that dideoxytetrosynol A inhibited the growth and induced apoptosis.

Recently, it has been suggested that telomerase might be an important factor in the proliferative capacity that is important for both cellular immortalization and carcinogenesis. The important role of telomerase in replicative senescence and the specific expression of this enzyme in most cancers raise the potential use of telomerase inhibition for cancer therapy. A majority of immortal and cancer cells have an indefinite proliferative capacity and maintain their telomere length by upregulating telomerase activity [37]. Bodnar *et al*. reported that ectopic expression of telomerase has been shown to extend the life-span of several normal human cells. Moreover, ectopic expression of hTERT in combination with oncogenes has been shown to be sufficient to convert normal human epithelial and fibroblast cells to tumor cells [15]. According to the telomere hypothesis for replicative senescence, inhibition of telomerase in the malignant cells is anticipated to result in the erosion of telomeres and ultimately leading to growth arrest, senescence or cell death. Indeed, inhibition of telomerase has been shown to limit the growth of human cancer cells in culture [5,7] supporting the potential use of telomerase inhibition for cancer therapy. As indicated in Fig. 5, dideoxytetrosynol A induced a concentration-dependent inhibition of telomerase activity and hTERT expression without altering the expression of hTR and TEP-1. Telomerase activity could be regulated by c-myc through controlling the expression of hTERT gene [1,7]. The ability of c-myc to function as a transcription factor has been shown to depend upon its dimerization with the protein Max [2]. However, in the present study, dideoxytetrosynol A treatment did not affect the c-myc levels (Fig. 4B). The results suggested that the repression of telomerase activity by dideoxytetrosynol A was associated with down-regulation of hTERT mRNA without alteration of c-myc expression.

In summary, the present study demonstrates that dideoxypetrosynol A, a polyacetylene from the marine sponge *Petrosia* sp., inhibited the U937 human monocytic leukamia cell proliferation by inducing G1 cell cycle arrest and apoptosis. Although further studies are needed, the present work suggests that p16 and pRB play an important role in G1 cell cycle arrest induced by dideoxypetrosynol A in human leukamia cells. The

apoptotic events of U937 cells by dideoxytetrosynol A were mediated by an increase in Bax expression and an activation of caspases. Dideoxytetrosynol A concomitantly causes a loss of PGE_2 and telomerase activity by decreasing the COX-2 and hTERT expression. These novel phenomena have not been previously described and provide important new insights into the possible biological effects of dideoxytetrosynol A. Although further studies are needed, the present work suggests that loss of COX-2 and telomerase activity may be a good surrogate biomarker for assessing anti-tumor activity of dideoxytetrosynol A.

Acknowledgement

This research was supported by a grant (B-2005-12) from Marine Bioprocess Research Center of the Marine Bio 21 Center funded by the Ministry of Maritime Affairs & Fisheries, Republic of Korea.

References

1. Biroccio, A. and Leonetti, C. 2004. Telomerase as a new target for the treatment of hormone-refractory prostate cancer. *Endocr. Relat. Cancer* **11**, 407-421.
2. Blackwood, E. M. and Eisenman, R. N. 1991. Max: a helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc. *Science* **251**, 1211-1217.
3. Bodnar, A. G., Ouellette, M., Frolkis, M. *et al*. 1998. Extension of life-span by introduction of telomerase into normal human cells. *Science* **279**, 349–352.
4. Cao, Y. and Prescott, S. M. 2002. Many actions of cyclooxygenase-2 in cellular dynamics and in cancer. *J. Cell. Physiol.* **190**, 279-286.
5. Chakraborty, S., Ghosh, U., Bhattacharyya, N. P. *et al*. 2006. Inhibition of telomerase activity and induction of apoptosis by curcumin in K-562 cells. *Mutat. Res*. **596**, 81-90.
6. Choi, H. J., Bae, S. J., Kim, N. D. *et al.* 2004. Induction of apoptosis by dideoxypetrosynol A, a polyacetylene from the sponge *Petrosia* sp., in human skin melanoma cells. *Int. J. Mol. Med.* **14**, 1091-1096.
7. Choi, S. H., Im, E., Kang, H. K. *et al*. 2005. Inhibitory effects of costunolide on the telomerase activity in human breast carcinoma cells. *Cancer Lett.* **227,** 153-162.
8. Crofford, L. J. 1997. COX-1 and COX-2 tissue expression: implications and predictions. *J. Rheumatol.* **49,** 15-19.
9. DeGregori, J., Leone, G., Ohtani, K. *et al*. 1995. E2F-1 accumulation bypasses a G1 arrest resulting from the inhibition of G1 cyclin-dependent kinase activity. *Genes Dev.* **9,** 2873-2887.
10. Dempke, W., Rie, C., Grothey, A. *et al*. 2001. Cyclooxygenase-2: a novel target for cancer chemotherapy? *J. Cancer Res. Clin. Oncol.* **127,** 411-417.
11. Deveraux, Q. L., Leo, E., Stennicke, H. R. *et al*. 1999. Cleavage of human inhibitor of apoptosis protein XIAP results in fragments with distinct specificities for caspases. *EMBO. J.* **18,** 5242–5251.
12. Earnshaw, W. C., Martins, L. M. and Kaufmann, S. H.. 1999. Mammalian caspases: structure, activation, substrates, and functions during apoptosis. *Annu. Rev. Biochem.* **68,** 383-424.
13. Elledges, S. J. and Harper, J. W. 1994. Cdk inhibitor: on the threshold of checkpoints and development. *Curr. Opin. Cell Biol.* **6,** 847-852.
14. Gately, S. and Kerbel, R. 2003. Therapeutic potential of selective cyclooxygenase-2 inhibitors in the management of tumor angiogenesis. *Prog. Exp. Tumor. Res.* **37,** 179-192.
15. Hahn, W. C., Counter, C. M., Lundberg, A. S., Beijersbergen, R. L., Brooks, M. W. and Weinberg, R. A. 1999. Creation of human tumour cells with defined genetic elements. *Nature* **400,** 464–468.
16. Johnson, D. G. and Schneider-Broussard, R. 1998. Role of E2F in cell cycle control and cancer. *Front. Biosci.* **3,** d447–d448.
17. Kim, D. K., Lee, M. Y., Lee, H. S. *et al*. 2002. Polyacetylenes from a marine sponge *Petrosia* sp. inhibit DNA replication at the level of initiation. *Cancer Lett.* **185,** 95-101.
18. Koff, A., Giordano, A., Desai, D. *et al*. 1992. Formation and activation of a cyclin E-cdk2 complex during the G1 phase of the human cell cycle. *Science* **272**, 1689-1694.
19. Lim, Y. J., Kim, J. S., Im, K. S. *et al*. New cytotoxic polyacetylenes from the marine sponge *Petrosia*. *J. Nat. Prod.* **62,** 1215-1217.
20. Li, Y., Nichols, M. A., Shay, J. W. *et al*. 1994. Transcriptional repression of the D-type cyclin-dependent kinase inhibitor p16 by the retinoblastoma susceptibility gene product pRb. *Cancer Res.* **54,** 6078–6082.
21. Loo, D. T. and Rillema, J. R. 1998. Measurement of cell death. *Methods Cell Biol.* **57,** 251-264.
22. Matsushime, H., Ewen, M. E., Strom, D. K. *et al*. 1992. Identification and properties of an atypical catalytic subunit (p34PSK-J3/cdk4) for mammalian D type G1 cyclins. *Cell* **71,** 323-334.
23. Mayer, A. M. and Gustafson, K. R. 2003. Marine pharmacology in 2000: antitumor and cytotoxic compounds. *Int. J. Cancer* **105,** 291-299.
24. McCarthy, P. J. Pitts, T. P., and Gunawardana, G. P. *et al*. 1992. Antifungal activity of meridine, a natural product from the marine sponge *Corticium* sp. *J. Nat. Prod.* **55,** 1664-1668.
25. Miyashita, T., Krajewski, S., Krajewska, M. *et al.* 1994. Tumor suppressor p53 is a regulator of bcl-2 and bax gene expression *in vitro* and *in vivo*. *Oncogene* 9, 1799-1805.
26. Morgan, D. 1997. Cyclin dependent kinases: Engines,

clocks and microprocessors. *Annu. Rev. Cell Dev. Biol.* **13,** 261–291.
27. Moroy, T. and Geisen, C. 2004. Cyclin E. *Int. J. Biochem. Cell Biol.* **36,** 1424-1439.
28. Newman, D. J. and Cragg, G. M. 2004. Marine natural products and related compounds in clinical and advanced preclinical trials. *J. Nat. Prod.* **67,** 1216-1238..
29. Oshima, M. and Taketo, M. M. 2002. COX selectivity and animal models for colon cancer. *Curr. Pharm. Des.* **8**, 1021-34.
30. Randazzo, A., Bifulco, G., Giannini, C. *et al*. 2001. Halipeptins A and B: two novel potent anti-inflammatory cyclic depsipeptides from the Vanuatu marine sponge *Haliclona* species. *J. Am. Chem. Soc.* **123,** 10870-10876.
31. Rosse, T., Olivier, R., Monney, L. *et al*. 1998. Bcl-2 prolongs cell survival after Bax-induced release of cytochrome C. *Nature* **391,** 496-499.
32. Roy, N., Deveraux, Q. L., Takahashi, R. *et al*. 1997. The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. *EMBO. J.* **16,** 6914–6925.
33. Sakaguchi, M., Fujii, Y., Hirabayashi, H. *et al*. 1996. Inversely correlated expression of p16 and Rb protein in non-small cell lung cancers: an immunohistochemical study. *Int. J. Cancer* **65,** 442–445.
34. Salomons, G. S., Brady, H. J., Verwijs-Janssen, M. *et al*. 1997. The Bax alpha:Bcl-2 ratio modulates the response to dexamethasone in leukaemic cells and is highly variable in childhood acute leukaemia. *Int. J. Cancer* **71,** 959-965.
35. Sandal, T. 2002. Molecular aspects of the mammalian cell cycle and cancer. *Oncologist.* **7,** 73-81.
36. Saraste, A. 1999. Morphologic criteria and detection of apoptosis. *Herz.* **24,** 189-95.
37. Shay, J. W. and Bacchetti, S. 1997. A survey of telomerase activity in human cancer. *Eur. J. Cancer* **33,** 787–91.
38. Tewari, M., Quan, L. T., O'Rourke, K. *et al*. 1995. Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. *Cell* **81**: 801-9.
39. Walker, P. R. and Sikorska, M. 1997. New aspects of the mechanism of DNA fragmentation in apoptosis. *Biochem. Cell Biol.* **75,** 287-99.
40. Weinberg, R. A. 1995. The retinoblastoma protein and cell cycle control. *Cell* **81,** 323-330.