Biochemical Pharmacology, Vol. 59, pp. 1109–1116, 2000.

ISSN 0006-2952/00/$–see front matter
PII S0006-2952(00)00235-5

Induction of G_1 Cell Cycle Arrest and $p27^{KIP1}$ Increase by Panaxydol Isolated from *Panax ginseng*

Jungae Moon, Su-Jin Yu,* Hyeon-Soo Kim*† and Jeongwon Sohn*†‡§*

*Graduate School of Biotechnology, Korea University †Department of Biochemistry, College of Medicine, Korea University and the ‡Medical Science Research Center, Korea University, Seoul, Korea.

ABSTRACT. Polyacetylenic compounds of *Panax ginseng* roots have been shown to inhibit growth of several human malignant tumor cell lines. Panaxydol is known to be one of the cytotoxic polyacetylenic compounds of *P. ginseng*. In this study, we first showed that panaxydol decreased markedly the proliferation, and to a lesser extent, the number of cells in a human melanoma cell line, SK-MEL-1. Next, the effect of panaxydol on cell cycle progression and its mechanism of action were investigated. Cell cycle analysis revealed that panaxydol inhibited cell cycle progression of a human malignant melanoma cell line, SK-MEL-1, at G_1-S transition. At the same time, panaxydol increased the protein expression of $p27^{KIP1}$ as early as 1 hr after treatment. Cyclin-dependent kinase 2 (Cdk2) activity was decreased in a dose-dependent manner after 24 hr of panaxydol treatment. Protein levels of $p21^{WAF1}$, $p16^{INK4a}$, p53, pRb (retinoblastoma protein), and E2F-1 were not changed. It was also found that cycloheximide reversed the growth inhibition induced by panaxydol and partially abrogated the increase in $p27^{KIP1}$ expression. These results indicate that panaxydol induces G_1 cell cycle arrest by decreasing Cdk2 activity and up-regulating $p27^{KIP1}$ protein expression. BIOCHEM PHARMACOL **59**;9: 1109–1116, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. panaxydol; cell cycle; G_1 arrest; $p27^{KIP1}$; Cdk2; melanoma

Panax ginseng C. A. Meyer is a herbal root that has been used in clinical practice for more than 2000 years throughout Far Eastern countries including China, Korea, and Japan. In general, ginseng has been traditionally known to increase arousal, stamina, and resistance to stress [1, 2]. Recently, many researchers have investigated the biological and pharmacological effects of *P. ginseng*, and there is a report that ginseng also possesses chemopreventive and therapeutic activities against cancers as well as improving the general immune function [3]. However, most studies on the pharmacological activities of *P. ginseng* have employed water-soluble components such as glycosides called ginseng saponins or ginsenosides. The petroleum ether extract of *P. ginseng* (GX-PE)‖ has been reported to possess antiproliferative effects on various cancer cell lines including murine sarcoma, murine leukemia, human colon carcinoma, and human renal cell carcinoma cell lines [4, 5]. Polyacetylenic compounds such as panaxynol, panaxydol, and panaxytriol have been reported to be responsible for these effects of GX-PE [6, 7]. However, little is known about the mechanism of their antiproliferative effect.

Cell cycle progression is regulated at several irreversible transition points, passage through which is controlled by the activity of Cdks [8, 9]. The activity of Cdk is regulated by at least three different mechanisms, i.e. binding of cyclin proteins, phosphorylation, and binding of CKIs. Progression from G_1 to S phase in mammalian cells is promoted by the accumulation of cyclins D, E, and A, which bind to and activate different Cdk catalytic subunits [10]. When mitogenic signals stimulate a cell to enter the cell cycle, the expression of D-type cyclins (cyclins D1, D2, and D3) is stimulated and maintained throughout G_1 as long as the growth factor is present. The D-type cyclins form complexes with either Cdk4 or Cdk6. These activated complexes then phosphorylate the Rb protein. Phosphorylation of pRb causes release of bound transcription factors, such as E2F-1, which then activate the transcription of genes whose products are required for entry into S phase of the cell cycle. Cyclin E is synthesized later than D-type cyclins and peaks late in G_1. The cyclin E/Cdk2 complex plays a role in G_1-S transition and the initiation of DNA synthesis. The complex of cyclin A and Cdk2 is thought to function in both the initiation of DNA synthesis and the progression through S phase. The transition from G_2 to M is driven by cdc2/cyclin B complexes.

Cdk activity is also regulated by both positive and negative phosphorylation events [11] as well as by associa-

§ Corresponding author: Jeongwon Sohn, M. D., Ph. D., Associate Professor, Department of Biochemistry, Korea University College of Medicine, 126-1 Anam-dong 5 Ga, Sungbuk-Gu, Seoul 136-705, Korea. Tel. 822 9206192; FAX 822 9230480; E-mail: biojs@kuccnx.korea.ac.kr

‖ *Abbreviations:* Cdk, cyclin-dependent kinase; GX-PE, petroleum ether extract of *Panax ginseng*; CKI, Cdk inhibitor; pRb, retinoblastoma protein; CHX, cycloheximide; and ActD, actinomycin D.

Received 17 February 1999; accepted 25 October 1999.

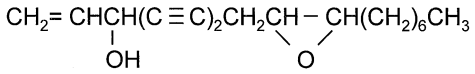

FIG. 1. Structure of panaxydol.

tion with inhibitory proteins [12, 13]. So far, two groups of CKIs have been identified in mammalian cells. One group includes $p21^{WAF1}$, $p27^{KIP1}$, and $p57^{KIP2}$, which bind to all G_1 cyclin/Cdk complexes [14–16], while the second includes $p15^{INK4b}$, $p16^{INK4a}$, $p18^{INK4c}$, and $p19^{INK4d}$, which specifically inhibit cyclin D/Cdk4 or cyclin D/Cdk6 [17, 18].

In a previous study, we reported that GX-PE inhibited the proliferation and cell cycle progression of three human melanoma cell lines [19]. Since GX-PE is a crude extract and thus not suitable for investigating the molecular mechanism of growth arrest, we decided in this study to use panaxydol (Fig. 1), one of the major cytotoxic compounds of GX-PE. The present study was aimed at examining if panaxydol suppresses proliferation of SK-MEL-1 by selectively perturbing cell cycle-related events. Our results show that panaxydol induces cell cycle arrest at G_1-S transition in SK-MEL-1 cells. This effect is associated with decreased Cdk2 activity, which seems to be caused by the increased level of $p27^{KIP1}$ protein expression. Growth inhibition by panaxydol requires new protein synthesis.

MATERIALS AND METHODS

Preparation of Panaxydol

Steamed and dried roots of 6-year-old *P. ginseng* (red ginseng) were purchased from the Korea Tobacco and Ginseng Company. Powdered red ginseng was extracted with petroleum ether (Mallinckrodt) at 40°. This extract was filtered through a 0.22-μm filter membrane and concentrated with a vacuum evaporator, and the residual petroleum ether was removed by N_2 gas. Panaxydol was purified from GX-PE through silicic acid column chromatography. Silicic acid (Merck) suspended in chloroform was poured into a column, and chloroform (Merck) was allowed to drain just to the top of the packed silicic acid. Then, the GX-PE was applied and the column was washed with petroleum ether and sequentially eluted with 9:1, 8:2, 7:3, and 6:4 mixtures of petroleum ether and ethyl ether (Junsei). Fractions containing panaxydol were identified by silica gel TLC. TLC plates (Merck) were baked for 2 hr at 120°, and 25–100 μg each of silicic acid column fractions were applied. After developing in petroleum ether/ethyl ether/acetic acid (90:10:1, v/v/v), spots were located by spraying 30% H_2SO_4 and drying the plate at 120° for 5 min. Fractions containing panaxydol were collected, aliquoted, and stored at −70°. Figure 2 shows the TLC analysis of GX-PE and the purified panaxydol. Before use, panaxydol was dissolved in absolute ethanol (Merck) and diluted in the culture medium so that the final concentration of ethanol would not exceed 0.1%. At the concentrations used in these experiments, ethanol itself did not have any effects on proliferation, cell numbers, or the activity of Cdk2.

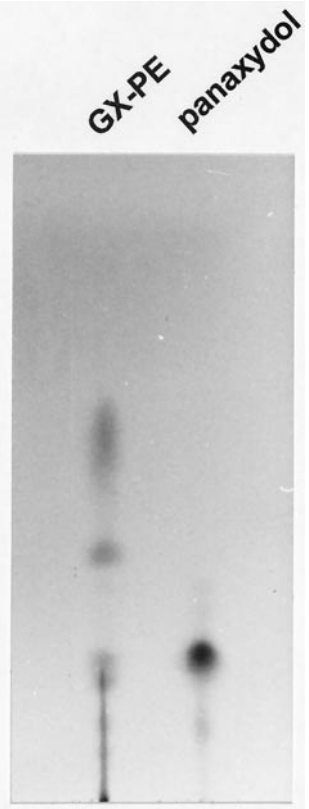

FIG. 2. Silica gel TLC of GX-PE and panaxydol. TLC was performed as described in 'Materials and Methods'.

Cell Culture

The human melanoma cell line SK-MEL-1 was obtained from the Korean Cell Line Bank. Cells were grown in RPMI-1640 (GIBCO BRL) supplemented with 10% fetal bovine serum (FBS), 100 U/mL of penicillin, 100 μg/mL of streptomycin, and 10 mM HEPES. Cells were maintained at 37° in humidified air containing 5% CO_2.

[^{3}H]Thymidine Incorporation Assay

Cells were plated on a 96-well plate at a density of 1×10^4 cells/well and treated with panaxydol as indicated. Six hours before the end of incubation, 1 μCi of [^{3}H]thymidine (Amersham) per well was added. Cells were harvested onto a glass fiber filter (Skatron). [^{3}H]thymidine incorporation into the DNA was measured with a β-scintillation counter (1214 Rackbeta, LKB). After the cells had been treated with panaxydol for 1 hr, the medium was replaced by a new one without panaxydol. Cells were cultured for a further 23 hr before analyses. Addition of [^{3}H]thymidine and measuring its incorporation were performed in the same way as in the continous 24-hr treatment.

Cell Cycle Analysis

Cells were harvested, washed with PBS, and fixed in 70% ethanol at −20° overnight. Cells were washed and suspended in PBS containing 200 μg/mL of RNase A (Sigma) and 20 μg/mL of propidium iodide (Sigma) and incubated for 30 min. The DNA content was measured by flow cytometry. The cell cycle was analyzed with the Cell Quest program (Becton Dickinson).

Immunoblot Analysis

Cells were lysed in 2 × sample buffer (250 mM Tris–HCl, pH 6.8, 4% SDS, 10% glycerol, 2% β-mercaptoethanol, 0.2 mM phenylmethylsulfonyl fluoride, 1 μg/mL of pepstatin A, 1 μg/mL of leupeptin, 1 μg/mL of aprotinin). Whole cell lysates containing 30 μg of proteins were boiled for 5 min, analyzed by SDS–PAGE, and electrotransferred onto a nitrocellulose membrane (Schleicher & Schuell). The membrane was blocked with 5% dried milk in Tris-buffered saline, 0.1% Tween 20 (TBS-T) for 1 hr, and incubated with a primary antibody for 1 hr. After washing in TBS-T, the membrane was incubated for 1 hr with an appropriate secondary antibody conjugated with horseradish peroxidase. Protein bands of interest were detected by enhanced chemiluminescence (ECL Western blotting kit, Amersham).

Immunoprecipitation and Cdk2 Assay

Cells were lysed in RIPA buffer (1% Triton X-100, 0.5% Nonidet P-40, 150 mM NaCl, 10 mM Tris–HCl, pH 7.4, 1 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 1 μg/mL of pepstatin A, 1 μg/mL of leupeptin, 1 μg/mL of aprotinin). Lysates were pre-cleared with Protein A–Sepharose (PAS) 4B (Amersham). The anti-Cdk2 antibody (M2, Santacruz) was added to the pre-cleared lysate and incubated overnight at 4°. PAS 4B was added the next day and the mixture was incubated for 1 hr at 4° and centrifuged. The pellets were washed several times with RIPA buffer and resuspended in kinase buffer (10 mM Tris–HCl, pH 7.4, 150 mM NaCl, 10 mM $MgCl_2$, 0.5 mM dithiothreitol) containing 5 μCi [γ-^{32}P]ATP (Amersham) and histone H1 (Sigma). After incubation for 20 min at 37°, the reaction was terminated by adding 10 μL of SDS-sample buffer (2×). Samples were boiled for 5 min, loaded on a 12% SDS polyacrylamide gel, and subjected to electrophoresis. Gels were stained with Coomassie blue and, after drying, analyzed by a phosphorimager (BAS-2500, Fujifilm).

RESULTS

Inhibition of SK-MEL-1 Proliferation by Panaxydol

To test whether panaxydol suppresses the growth of a human melanoma cell line, SK-MEL-1, cells were treated with panaxydol at different concentrations for 24 hr, and proliferation was assayed by [^{3}H]thymidine incorporation assay. As shown in Fig. 3A, panaxydol strongly inhibited proliferation of SK-MEL-1 cells in a dose-dependent manner. When cells were treated with panaxydol continuously for 24 hr, the growth of cells was inhibited by 54.5% at a concentration of 10 μg/mL and by 86.3% at 20 μg/mL. When cells were treated with panaxydol for 1 hr and further incubated for 23 hr without panaxydol, inhibition of proliferation was also observed albeit to a smaller degree (38.2% at 100 μg/mL) (Fig. 3B). The number of viable cells was also counted. The viable cell number was reduced by up to 36% (at 30 μg/mL of panaxydol) compared to the 92.8% reduction in proliferation under the same conditions (Fig. 3A). Since the decreased cell number can be explained, at least in part, by the inhibition of proliferation, the most prominent effect of panaxydol at 24 hr of treatment seems to be cytostatic rather than cytotoxic. Ethanol itself did not have any effect on proliferation or cell number at the concentrations used in these experiments. These results show that panaxydol directly inhibits proliferation of a human melanoma cell line, SK-MEL-1.

Induction of G_1 Cell Cycle Arrest by Panaxydol

In order to examine the mechanism of growth suppression by panaxydol, SK-MEL-1 cells treated with panaxydol for 24 hr were analyzed for cell cycle by flow cytometry. Panaxydol altered the cell cycle distribution of the asynchronously growing SK-MEL-1 cells (Fig. 4). SK-MEL-1 cells incubated with panaxydol contained reduced S and increased G_1 phase populations compared to untreated control cells. As seen in Table 1, the effect of panaxydol on the cell cycle was dose-dependent. These results suggest that panaxydol suppresses cell growth by inducing a specific block at G_1-S transition of the cell cycle. Data shown in Table 1 and those from separate experiments (not shown) indicate that progression through G_2 phase is not affected by panaxydol.

Effect of Panaxydol on the Expression of a CKI, $p27^{KIP1}$

Since panaxydol arrests the cell cycle at G_1 phase, we investigated whether panaxydol selectively regulates the expression of G_1-specific cell cycle regulators. To identify any cell cycle regulators that are controlled by panaxydol, expression levels of $p21^{WAF1}$, $p27^{KIP1}$, $p16^{INK4a}$, p53, pRb, and E2F-1 were examined by immunoblot analyses. Among these proteins, only $p27^{KIP1}$ was significantly increased in a time- and dose-dependent manner (Fig. 5). The level of $p27^{KIP1}$ started to increase as early as 1 hr after panaxydol treatment. Protein levels of pRb, $p21^{WAF1}$, p53, $p16^{INK4a}$, and E2F-1 remained unchanged, nor was there a change in the phosphorylation status of pRb (data not shown). These results suggest that panaxydol specifically induces $p27^{KIP1}$ expression.

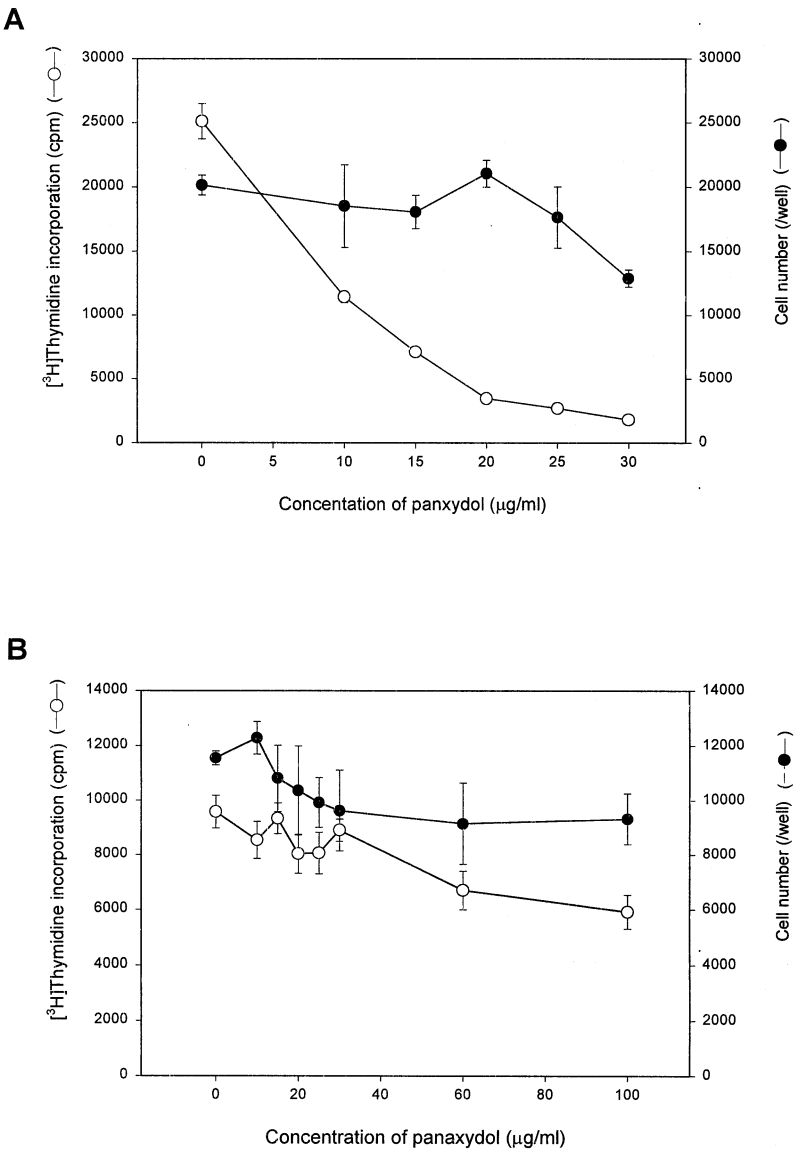

FIG. 3. Effect of panaxydol on the proliferation and cell number of SK-MEL-1. (A) Cells were treated with various concentrations of panaxydol for 24 hr for the proliferation assay. During the last 6 hr of incubation, [^{3}H]thymidine was added to the culture. Incorporation of [^{3}H]thymidine into the newly synthesized DNA was measured with a β-scintillation counter (open circles). Similarly, cells were treated with panaxydol for 24 hr and the number of cells was counted (closed circles). The reported values are means ± SE of triplicate determinations. (B) Cells were treated with various concentrations of panaxydol for 1 hr, washed, and incubated in a fresh medium without panaxydol for 23 hr. Addition of [^{3}H]thymidine and measuring its incorporation were carried out as in (A) (open circles). Similarly, cells were treated with panaxydol for 1 hr, washed, and incubated in a fresh medium without panaxydol for 23 hr, and the number of cells was counted (closed circles). The reported values are means ± SE of triplicate determinations.

Effect of Panaxydol on Cdk2 Activity

Transit through G_1 and entry into S phase require the actions of Cdks such as Cdk2, Cdk4, and Cdk6. These G_1 Cdks can be inactivated by Cdk inhibitors such as $p27^{KIP1}$. $p27^{KIP1}$ is known to be directly involved in a restriction point control and to inhibit cyclinE/Cdk2 and cyclinA/Cdk2 activity in cycling cells. Since panaxydol blocked the cell cycle at G_1-S transition stage and the protein level of $p27^{KIP1}$ was increased, the activity of Cdk2 in SK-MEL-1 cells treated with panaxydol was analyzed. As shown in Fig. 6A, the activity of Cdk2 was decreased in a dose-dependent manner in response to panaxydol at 24 hr. To rule out the possibility that the decrease in Cdk2 activity was due to ethanol which was used as a vehicle, ethanol was added so that the final concentration was the same in all samples, including the control. The dose-dependent decrease in Cdk2 activity when the concentration of ethanol was the same in all samples demonstrated that the effect was due to panaxydol and not the vehicle, ethanol. Analysis of Cdk2 activity at different times showed that panaxydol decreased Cdk2 activity at 24 hr (Fig. 6B). Panaxydol-induced reduction in Cdk2 activity was not observed up to 8 hr. These results indicate that the G_1 cell cycle arrest induced by panaxydol is associated with the decrease in Cdk2 activity.

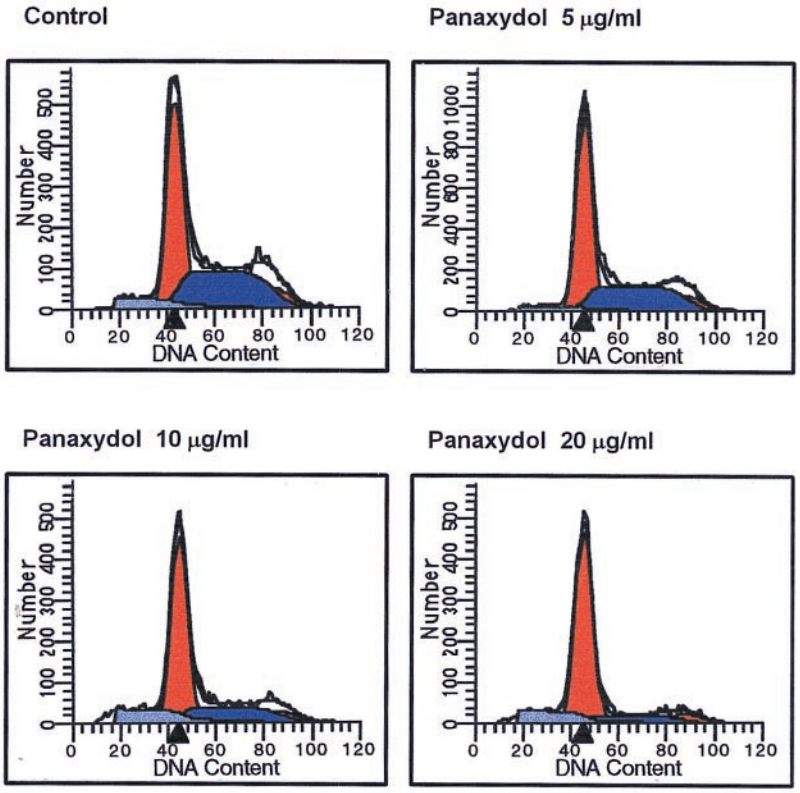

FIG. 4. Cell cycle analysis of SK-MEL-1 treated with panaxydol. Cells were treated for 24 hr with panaxydol at indicated concentrations. After the cells were fixed and stained with propidium iodide, the DNA content was measured by flow cytometry. Cell cycle distribution was analyzed with the Cell Quest program (Becton Dickinson).

Effect of CHX as an Inhibitor of Protein Synthesis and ActD as an Inhibitor of Transcription

To determine whether panaxydol requires new RNA and/or protein synthesis to exert its growth inhibitory effect, SK-MEL-1 cells were treated with panaxydol in the presence or absence of CHX or ActD. Since the protein level of $p27^{KIP1}$ is increased by panaxydol, the effect of CHX on $p27^{KIP1}$ expression was examined. Immunoblot analysis revealed that CHX partially abrogated the increase in the $p27^{KIP1}$ protein (Fig. 7). This result shows that the increase in $p27^{KIP1}$ is, in part, due to new protein synthesis. The proliferation assay showed that CHX also reduced the antiproliferative effect of panaxydol. From Fig. 8A (3^{rd} and 4^{th} bars), we calculated that growth inhibition by panaxydol (80 μg/mL) was decreased by about 36% when CHX was added. A similar extent of reduction in panaxydol-induced growth inhibition was also observed when 100 μg/mL of panaxynol was used (5^{th} and 6^{th} bars of Fig. 8A). However, ActD had no obvious effect on the antiproliferative activity of panaxydol. Since ActD was highly toxic to SK-MEL-1 cells, we could not test the effect of ActD at concentrations higher than 20 ng/mL. Thus, the antiproliferative effect of panaxydol may require new protein synthesis, but not *de novo* RNA synthesis. Reversal of both the panaxydol-induced growth inhibition and the increase in $p27^{KIP1}$ protein expression by CHX suggests that panaxydol induces G_1 cell cycle arrest by increasing $p27^{KIP1}$ expression.

DISCUSSION

Here, we demonstrated that panaxydol from *P. ginseng* roots inhibited *in vitro* proliferation of a human melanoma cell line, SK-MEL-1. Most drugs which inhibit cell cycle pro-

TABLE 1. Cell cycle distribution of SK-MEL-1 cells treated with varying concentrations of panaxydol for 24 hr

Panaxydol (μg/mL)	Cell cycle distriution (%)			
	G_0/G_1	S	G_2/M	G_0/G_1:S ratio
0	47.70	47.44	4.86	1.01
5	55.54	36.11	8.35	1.54
10	64.99	27.41	7.60	2.37
20	78.46	13.98	7.57	5.61

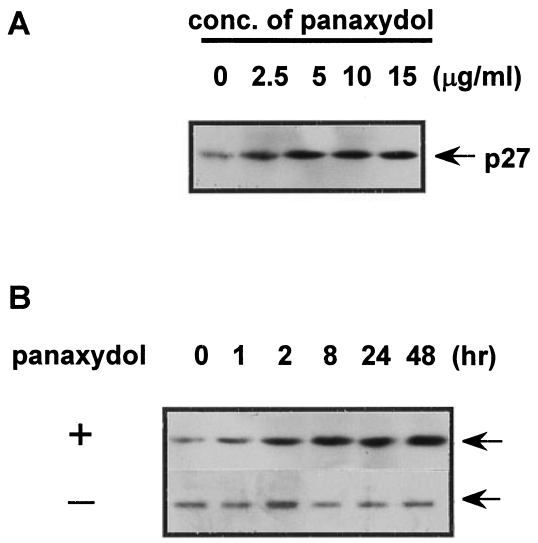

FIG. 5. Effect of panaxydol on the protein level of $p27^{KIP1}$. SK-MEL-1 cells were treated with various concentrations of panaxydol for 24 hr (A) or with 10 μg/mL of panaxydol for indicated times (B). The protein expression of $p27^{KIP1}$ was determined by immunoblot analysis.

gression exert their effects at G_1 and/or G_2 stage(s) [20, 21]. Cell cycle analysis showed that panaxydol arrested the cell cycle at the G_1-S transition in a dose-dependent manner. Panaxynol did not affect progression through G_2 stage.

Activities of many cyclin/Cdk complexes can be controlled by Cdk inhibitors. In mammalian cells, two classes of CKIs, i.e. the CIP/KIP and Ink4 families, provide a tissue-specific mechanism by which cell cycle progression can be restrained in response to extracellular and intracellular signals [22, 23]. $p27^{KIP1}$ is a member of the CIP/KIP family. It associates with complexes of cyclins D1–3/Cdk4, cyclins D1–3/Cdk6, cyclin E/Cdk2, and cyclin A/Cdk2 [24, 25]. However, $p27^{KIP1}$ inhibits cyclin E/Cdk2 more effectively than cyclin A/Cdk2 or cyclin D/Cdk4 [15] and thus plays an important role in regulation of passage through late G_1 phase. In various cellular systems examined previously, $p27^{KIP1}$ levels decrease following mitogenic stimulation of quiescent cells, or conversely, increase following contact inhibition, mitogen withdrawal, or other antiproliferative signals [26–29].

Immunoblot analyses demonstrated that panaxydol increased the protein level of $p27^{KIP1}$ concomitantly with G_1 cell cycle arrest in SK-MEL-1 cells. However, expression levels of $p21^{WAF1}$, pRb, $p16^{INK4a}$, E2F-1, and p53 were not changed. Cdk2 activity was significantly decreased at 24 hr of panaxydol treatment in a dose-dependent manner. These data suggest that panaxydol induces G_1 cell cycle arrest by inhibiting Cdk2 activity and that the decrease in Cdk2 activity may be due to increased $p27^{KIP1}$ expression.

It has been reported in some cell types that extracellular signals induce changes in total $p27^{KIP1}$ levels [23] by either controlling translation or ubiquitin-dependent degradation.

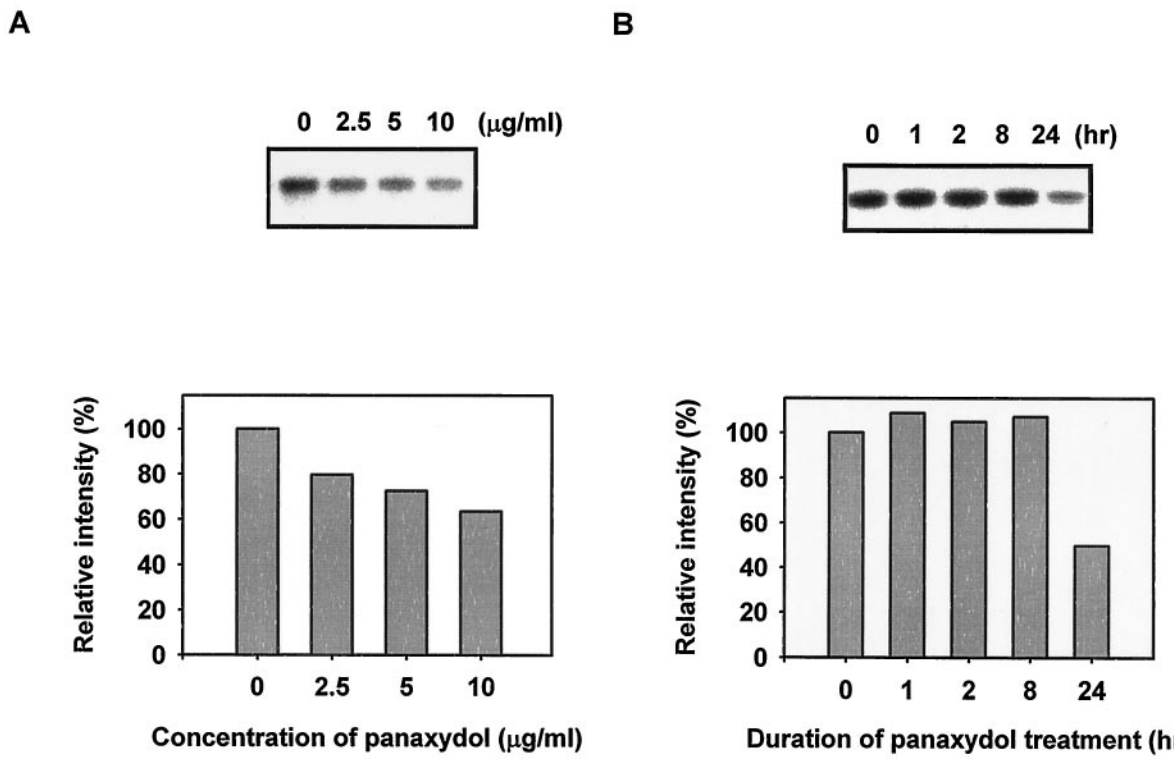

FIG. 6. Cdk2 activity in SK-MEL-1 treated with panaxydol. Cells were treated with various concentrations of panaxydol for 24 hr (A) and 5 μg/mL of panaxydol for indicated times (B). Cdk2 was immunoprecipitated from cell lysates and assayed for *in vitro* kinase activity using histone H1 as a substrate. The kinase reaction mixtures were separated by SDS–PAGE and the level of [^{32}P]-labeled histone was analyzed by phosphorimager (upper panels). The intensity of each band was quantified by densitometry (lower panels).

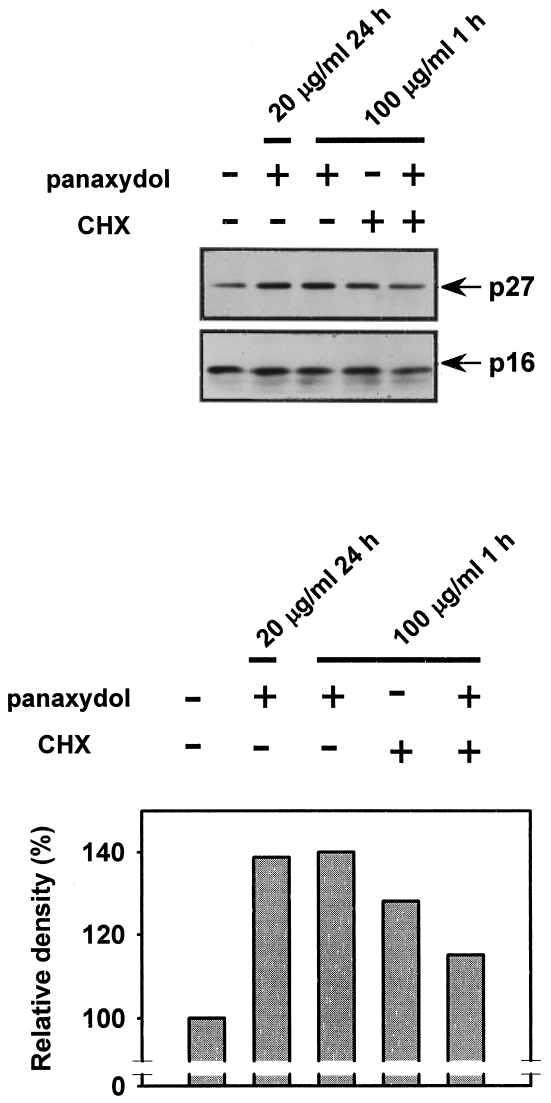

FIG. 7. Effect of CHX on $p27^{KIP1}$ induction by panaxydol. Where CHX was added, cells were pretreated with 10 μg/mL of CHX for 1 hr before panaxydol was added. After a further incubation of 1 hr in the presence of panaxydol, cells were washed and incubated in a fresh medium without panaxydol and CHX. Following 23-hr incubation, cells were harvested for an immunoblot analysis (upper panel). For comparison, $p27^{KIP1}$ expression in cells treated with 20 μg/mL of panaxydol continuously for 24 hr was analyzed in parallel (the 2nd lane). The intensity of each band was quantified by densitometry (lower panel).

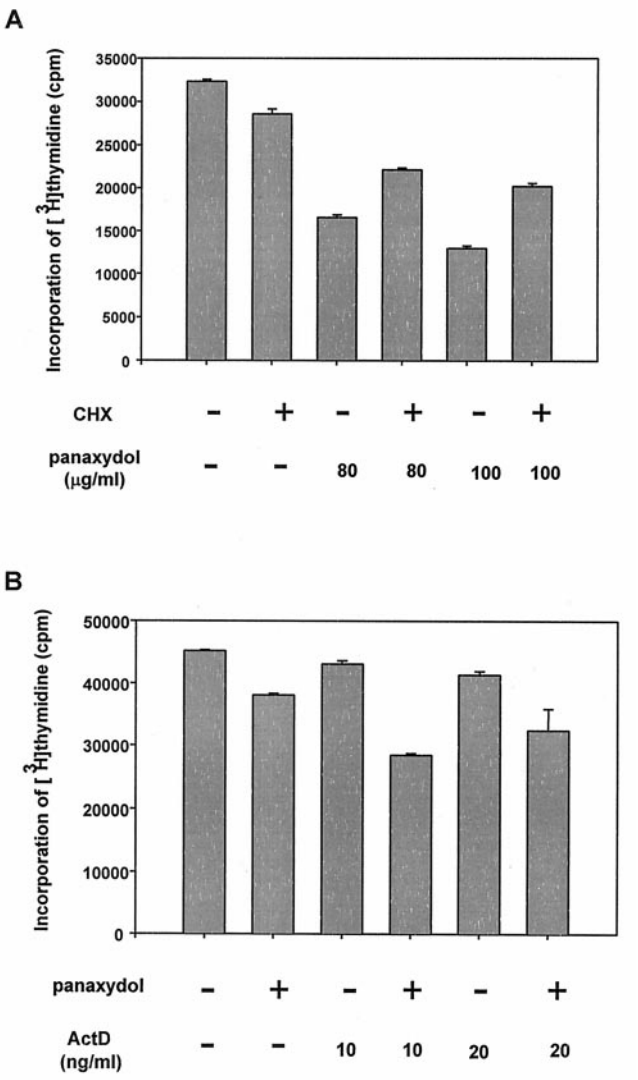

FIG. 8. (A) Effects of CHX on the growth inhibition of SK-MEL-1 by panaxydol. Cells were pretreated with CHX (10 μg/mL) for 1 hr before panaxydol was added. After 1 hr of panaxydol treatment (80 or 100 μg/mL), cells were washed and incubated in a fresh medium without panaxydol and CHX for 23 hr. For the last 6 hr of incubation, cells were labeled with [^{3}H]thymidine. The incorporation of [^{3}H]thymidine into the newly synthesized DNA was determined with a β-scintillation counter. The reported values are means ± SE of triplicate determinations. (B) Effects of ActD on the growth inhibition of SK-MEL-1 by panaxydol. Cells were incubated with ActD (10 or 20 ng/mL) for 1 hr before panaxydol was added. After 1 hr of panaxydol treatment (100 μg/mL), cells were washed and incubated in a new medium without panaxydol and ActD for 23 hr. Addition of [^{3}H]thymidine and measuring its incorporation were carried out as in (A). The reported values are means ± SE of triplicate determinations.

A rapid increase in translation of $p27^{KIP1}$ may be essential for negative regulation of G_1 progression in response to antiproliferative signals [29]. On the other hand, $p27^{KIP1}$ is degraded by the ubiquitin pathway in both normal and transformed mammalian cells. Compared with proliferating cells, quiescent cells exhibited a smaller amount of $p27^{KIP1}$ ubiquitinating activity [30]. Degradation of $p27^{KIP1}$ by proteasome-mediated proteolysis has recently been reported in aggressive colorectal carcinomas and non-small cell lung cancer [30, 31]. Thus, the increased level of $p27^{KIP1}$ by panaxydol can be achieved by regulation of either increased translation or decreased ubiquitin-dependent degradation.

In this study, a protein synthesis inhibitor, CHX, reversed the growth suppression induced by panaxydol (80 μg/mL) by about 36%. At the same time, CHX partially prevented the increase in $p27^{KIP1}$ expression. Although we did not test the degradation rate of $p27^{KIP1}$ protein, the increase in $p27^{KIP1}$ by panaxydol was apparently due to the enhanced protein synthesis, since CHX could block the

increase. These results demonstrate that panaxydol affected cellular proliferation, in part by increasing translation of $p27^{KIP1}$. However, mechanisms other than regulation of protein synthesis are likely to be involved in the growth inhibition of SK-MEL-1 cells, since CHX did not reverse the growth inhibition caused by panaxynol completely.

We would like to thank Dr. Young Mie Ha-Lee for helpful discussions. This study was supported by a grant from the Medical Science Research Center, Korea University.

References

1. Brekhman II and Dardymov IV, New substances of plant origin which increase nonspecific resistance. *Annu Rev Pharmacol* **9:** 419–430, 1969.
2. Takagi K, Saito H and Nabata H, Pharmacological studies of *Panax ginseng* roots: Estimation of pharmacological actions of *Panax ginseng* root. *Jpn J Pharmacol* **22:** 245–249, 1972.
3. Xiaoguang C, Hongyan L, Xiaohong L, Zhaodi F, Yan L, Lihua T and Rui H, Cancer chemopreventive and therapeutic activities of red ginseng. *J Ethnopharmacol* **60:** 71–78, 1998.
4. Lee SH and Hwang WI, Inhibitory effect of petroleum ether extract of *Panax ginseng* root against growth of human cancer cells. *Korean J Ginseng Sci* **10:** 141–150, 1986.
5. Sohn J, Lee CH, Chung DJ, Park SH, Kim I and Hwang WI, Effect of petroleum ether extract of *Panax ginseng* roots on proliferation and cell cycle progression of human renal cell carcinoma cells. *Exp Mol Med* **30:** 47–51, 1998.
6. Saita T, Katano M, Matsunaga H, Yamamoto H, Fujito H and Mori M, The first specific antibody against cytotoxic polyacetylenic alcohol, panaxynol. *Chem Pharm Bull (Toyko)* **41:** 549–552, 1993.
7. Matsunaga H, Saita T, Nagamo F, Mori M and Katano M, A possible mechanism for the cytotoxicity of a polyacetylenic alcohol, panaxytriol: Inhibition of mitochondrial respiration. *Cancer Chemother Pharmacol* **35:** 291–296, 1995.
8. Sherr CJ, Cancer cell cycles. *Science* **274:** 1672–1677, 1996.
9. DeVita VT Jr, Hellman S and Rosenberg SA, Malignant melanoma. In: *Cancer: Principles & Practice of Oncology*, 5th Edn (Eds. Devita VT Jr and Hellman S), pp. 1935–1944. Lippincott-Raven, Philadelphia, 1997.
10. Draetta GF, Cell cycle: Will the real Cdk-activating kinase please stand up. *Curr Biol* **7:** R50–R52, 1997.
11. Coleman TR and Dumphy WG, Cdc2 regulatory factors. *Curr Opin Cell Biol* **6:** 877–882, 1994.
12. Peters G, Cell cycle. Stifled by inhibitions. *Nature* **371:** 204–205, 1994.
13. Sherr CJ and Roberts JM, Inhibitors of mammalian G_1 cyclin-dependent kinases. *Genes Dev* **9:** 1149–1163, 1995.
14. Slingerland JM, Hengst L, Pan CH, Alexander D, Stampfer MR and Reed SI, A novel inhibitor of cyclin–Cdk activity detected in transforming growth factor beta-arrested epithelial cells. *Mol Cell Biol* **14:** 3683–3694, 1994.
15. Polyak K, Lee MH, Erdjument-Bromage H, Koff A, Roberts JM, Tempst P and Massague J, Cloning of $p27^{KIP1}$, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals. *Cell* **78:** 59–66, 1994.
16. Lee MH, Reynisdottir I and Massague J, Cloning of $p57^{KIP2}$, a cyclin-dependent kinase inhibitor with unique domain structure and tissue distribution. *Genes Dev* **9:** 639–649, 1995.
17. Hannon GJ and Beach D, $p15^{INK4B}$ is a potential effector of TGF-beta-induced cell cycle arrest. *Nature* **371:** 257–261, 1994.
18. Guan KL, Jenkins CW, Li Y, Nichols MA, Wu X, O'Keefe CL, Matera AG and Xiong Y, Growth suppression by p18, a $p16^{INK4}$/MTS1- and $p14^{INK4B}$/MTs2-related CDK6 inhibitor, correlates with wild-type pRb function. *Genes Dev* **8:** 2939–2952, 1994.
19. Kim HJ, Roh JY, Choi JO, Park SH, Kim IS, Sohn J and Ryu CK, Effects of lipid-soluble components of Korean Red Ginseng on proliferation and cell cycle regulation factors in human melanoma cell lines. *J Korean Cancer Assoc* **29:** 965–976, 1997.
20. Lepley DM and Pelling JC, Induction of p21/WAF1 and G_1 cell-cycle arrest by the chemopreventive agent apigenin. *Mol Carcinog* **19:** 74–82, 1997.
21. Smith PJ, Soues S, Gottlieb T, Falk SJ, Watson JV, Osborne RJ and Bleehen NM, Etoposide-induced cell cycle delay and arrest-dependent modulation of DNA topoisomerase II in small-cell lung cancer cells. *Br J Cancer* **70:** 914–921, 1994.
22. Harper JW and Elledge SJ, Cdk inhibitors in development and cancer. *Curr Opin Genet Dev* **6:** 56–64, 1996.
23. Morgan DO, Cyclin-dependent kinases: Engines, clocks, and microprocessors. *Annu Rev Cell Dev Biol* **13:** 261–291, 1997.
24. Toyoshima H and Hunter T, p27, a novel inhibitor of G_1 cyclin–Cdk protein kinase activity, is related to p21. *Cell* **78:** 67–74, 1994.
25. Vlach J, Hennecke S and Amati B, Phosphorylation-dependent degradation of the cyclin-dependent kinase inhibitor $p27^{KIP1}$. *EMBO J* **16:** 5334–5344, 1997.
26. Coats S, Flanagan WM, Nourse J and Roberts JM, Requirement of $p27^{KIP1}$ for restriction point control of the fibroblast cell cycle. *Science* **272:** 877–880, 1996.
27. Nourse J, Firpo E, Flanagan WM, Coats S, Polyak K, Lee MH, Massague J, Crabtree GR and Roberts JM, Interleukin-2-mediated elimination of the $p27^{Kip1}$ cyclin-dependent kinase inhibitor prevented by rapamycin. *Nature* **72:** 570–573, 1994.
28. Hengst L and Reed SI, Translational control of $p27^{Kip1}$ accumulation during the cell cycle. *Science* **271:** 1861–1864, 1996.
29. Rivard N, L'Allemain G, Bartek J and Pouyssegur J, Abrogation of $p27^{Kip1}$ by cDNA antisense suppresses quiescence (G0 state) in fibroblasts. *J Biol Chem* **271:** 18337–18341, 1996.
30. Esposito V, Baldi A, De Luca A, Groger AM, Loda M, Giordano GG, Caputi M, Baldi F, Pagano M and Giordano A, Prognostic role of the cyclin-dependent kinase inhibitor p27 in non-small cell lung cancer. *Cancer Res* **57:** 3381–3385, 1997.
31. Loda M, Cukor B, Tam SW, Lavin P, Fiorentino M, Draetta GF, Jessup JM and Pagano M, Increased proteasome-dependent degradation of the cyclin-dependent kinase inhibitor p27 in aggressive colorectal carcinomas. *Nat Med* **3:** 231–234, 1997.