G1-checkpoint Function Including a Cyclin-dependent Kinase 2 Regulatory Pathway as Potential Determinant of 7-Hydroxystaurosporine (UCN-01)-induced Apoptosis and G1-phase Accumulation

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7-Hydroxystaurosporine (UCN-01), which was originally identified as a protein kinase C selective inhibitor, is currently in clinical trials as an anti-cancer drug. We previously showed that UCN-01 induced preferential G1-phase accumulation in tumor cells and this effect was associated with the retinoblastoma (Rb) protein and its regulatory factors, such as cyclin-dependent kinase 2 (CDK2) and CDK inhibitors p21Cip1/WAF1 and p27Kip1. We demonstrate here that G1-phase accumulation was induced by UCN-01 in Rb-proficient cell lines (WiDr and HCT116 human colon carcinomas and WI-38 human lung fibroblast), and it was accompanied by dephosphorylation of Rb. In addition, UCN-01-induced G1-phase accumulation was also demonstrated in a Rb-defective cell line (Saos-2 human osteosarcoma), but not in the other Rb-proficient cell lines. These observations suggest that G1-checkpoint function might be important for cell survival during UCN-01 treatment. In addition, there may be a UCN-01-responsive factor in the G1-checkpoint machinery other than Rb which is targeted by SV40. Further studies revealed a correlation between UCN-01-induced G1-phase accumulation and reduction of cellular CDK2 kinase activity. This reduction was strictly dependent on down-regulation of the Thr160-phosphorylated form of CDK2 protein, and coincided in part with up-regulation of p27Kip1, but it was independent of the level of the p21Cip1/WAF1 protein. These results suggest that G1-checkpoint function, including a CDK2-regulatory pathway, may be a significant determinant of the sensitivity of tumor cells to UCN-01.

Key words: 7-Hydroxystaurosporine (UCN-01) — G1-phase accumulation — Apoptosis — Cyclin-dependent kinase 2 — G1 checkpoint

7-Hydroxystaurosporine (UCN-01) was originally isolated from the culture broth of *Streptomyces* sp. as a protein kinase C (PKC)-selective inhibitor.1) UCN-01 has previously been shown to exhibit potent antitumor activity as a single agent and in combination with DNA-damaging agents and anti-metabolites in vitro and in vivo.2–4) In addition, the compound showed a unique fingerprint pattern, so-called “compare negative,” in the in vitro anti-cancer drugs screening panel of the National Cancer Institute in the USA.5) Because of these unique characteristics of the drug, UCN-01 is currently in clinical trials as an anticancer drug in the USA and Japan.6)–7)

Cell cycle progression in mammalian cells is regulated by members of a cyclin-dependent kinase (CDK)/cyclin family.8) Progression from G1 to S phase is positively regulated by CDK4/cyclin D and CDK2/cyclin E complexes through phosphorylation of their target, the retinoblastoma (Rb) protein. CDK4 and CDK2 need to be phosphorylated at Thr172 and Thr160 by CDK-activating kinase (CAK) for their activation. The G1 CDKs are negatively regulated by interaction with CDK inhibitor proteins. Previously, one of the CDK inhibitor proteins p21Cip1/WAF1 was proven to be induced by p53, indicating that p53 is one of the regulators of G1-phase progression.8) Recent evidence has revealed that the G1-phase regulators are often mutated or deleted in tumor cells and may be associated with the tumorigenic process.9) In addition, p53 and Rb proteins are targeted and inactivated by viral oncoproteins such as simian virus 40 (SV40) large T-antigen and human papillomavirus E6 and E7.10,11)

Apoptosis is a biological process of cell death defined by morphological phenomena such as chromatin condensation and cell shrinkage, and it is associated with various biochemical phenomena.12) A major event in the apoptotic process is activation of caspases, the activation of which leads to proteolysis of death substrates such as poly(ADP-ribose) polymerase (PARP), DNA-dependent protein kinase and caspases themselves.13) Apoptosis can be induced not only by physiological stimuli but also by artificial stress such as that linked to exposure to ionizing radiation and anti-cancer drugs.12)

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addition, we have recently shown that G1-phase accumulation induced by UCN-01 is associated with dephosphorylation of Rb protein by inhibition of CDK2 mediated through induction of CDK inhibitor proteins p21Cip1/WAF1 and p27Kip1 in A431 human epidermoid carcinoma cells. Other groups have reported that UCN-01 induces apoptosis in human leukemia and colon carcinoma cell lines and in rat thymocytes. Here, we examined the effect of UCN-01 on the cell cycle distribution and its regulatory proteins such as Rb, p21Cip1/WAF1, p27Kip1 and CDK2 in various types of human cell lines in order to elucidate the significance of the regulatory proteins for G1-phase accumulation induced by the drug.

MATERIALS AND METHODS

Reagent and antibodies  UCN-01 was produced by fermentation in our institute as described previously. The drug was dissolved in dimethylsulfoxide (DMSO), and freshly diluted with cell culture medium. Anti-Rb (sc-50), anti-p21Cip1/WAF1 (sc-397) and anti-p27Kip1 (sc-528) rabbit polyclonal antibodies were purchased from Santa Cruz (Santa Cruz, CA). Anti-PARP mouse monoclonal antibody was purchased from Enzyme Systems Products (Dublin, CA). Anti-CDK2 mouse monoclonal antibody was produced in our Tokyo Research Laboratories (Tokyo).

Cell culture  WI-38 (human lung fibroblast) cells were purchased from RIKEN Gene Bank (Ibaraki). DLD-1, WiDr and HCT116 (human colon carcinomas), Hep G2 (human hepatoma) and WI-38 VA13 (SV40 transformant) cells were purchased from Dainippon Pharmaceutical (Osaka). S637 (human bladder carcinoma) and Saos-2 (human osteosarcoma) cells were kindly provided by Dr. R. Takahashi (Kyoto University, Kyoto). A549 (human non-small cell lung cancer) cells were kindly provided by Dr. A. Gescher (University of Leicester, Leicester, UK). Cell culture media used were RPMI1640 (Gibco BRL, Rockville, MD) for T47D, MCF-7, DLD-1, S637 and Saos-2 cells, Dulbecco’s modified Eagle’s medium (Nissui, Tokyo) for Hep G2, WI-38 and WI-38 VA13 cells, minimum essential medium (MEM; Nissui) for WiDr cells, McCoy 5A (Dainippon Pharmaceutical) for HCT116 cells, and Ham’s F-12 (Gibco BRL) for A549 cells. These media contained 10% fetal bovine serum (Gibco BRL). RPMI1640 medium for MCF-7 cells was further supplemented with 10 nM 17β-estradiol (Sigma Chemical, St. Louis, MO). MEM medium was further supplemented with non-essential amino acids (Dainippon Pharmaceutical). The cell cultures were performed at 37°C in a humidified atmosphere of 5% CO2.

Cell growth assay  Cells (1.5–3×10⁴/well) were plated and cultured in appropriate medium in 96-well microtiter plates (Nunc, Roskilde, Denmark) overnight, and treated with UCN-01 for 72 h. Cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Cell cycle analysis  Cells (3–6×10⁴/dish) were cultured overnight in appropriate medium in Falcon 3003 plastic dishes (Becton Dickinson, Lincoln Park, NJ), and treated with UCN-01 for 24 h. The UCN-01-pretreated cells were washed with phosphate-buffered saline (PBS) and detached by treatment with 0.25% trypsin. The cells were fixed with ice-cold 70% ethanol solution for cell cycle analysis. Alternatively, the cells were frozen as a pellet at −80°C for western blot analysis or kinase assay. The ethanolfixed cells were hydrolyzed with 0.25 mg/ml RNase A (type 1-A, Sigma) at 37°C for 30 min, and stained with propidium iodide (PI; Sigma) for 20 min. The DNA content of the cells was analyzed by an EPICS ELITE flow cytometer (Coulter, Hialeah, FL), and the cell cycle distributions were visualized as DNA histograms.

Western blot analysis  Cells were lysed in lysis buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 0.1% Triton X-100, 50 mM NaF, 80 mM β-glycerophosphate, 0.1 mM Na3VO4, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 µg/ml aprotinin, 1 µg/ml leupeptin) for 20 min at 4°C. The cell lysate was clarified by centrifugation at 15,000g for 10 min at 4°C and its protein content was determined using the protein assay kit (Bio-Rad Laboratories, Hercules, CA). The whole cell lysate was heated in 1× sodium dodecyl sulfate (SDS)-sample buffer for 5 min at 95°C, and subjected to SDS-polyacrylamide gel electrophoresis. The protein was transferred to a nitrocellulose membrane (Bio-Rad), probed with primary antibodies followed by secondary antibodies conjugated with horseradish peroxidase (Amersham, Buckinghamshire, UK), and detected by the enhanced chemiluminescence system (Amersham).

Immunoprecipitation and kinase assay  Whole cell lysate was prepared and its protein content was determined as described above. Then 400 µg of protein was added to 500 µl of protein A-Sepharose CL-4B (4% v/v, Pharmacia, Uppsala, Sweden) preassociated with anti-CDK2 antibody, and mixed gently for 2 h at 4°C. The immunoprecipitate was washed with lysis buffer twice and wash buffer (50 mM HEPES, pH 7.4, 10 mM MgCl2, 1 mM diithiothreitol (DTT)) twice. Each CDK2-immunoprecipitate was mixed with 40 µl of wash buffer containing 16 µg of histone H1 (Boehringer-Mannheim, Mannheim, Germany), 50 µM adenosine 5′-triphosphate (ATP) and 2.5 µCi of [γ-32P]ATP (5000 Ci/mmol, Amersham), and incubated for 10 min at 30°C. Each sample was mixed with 30 µl of 3× SDS-sample buffer to stop the reaction, heated for 5 min at 95°C and subjected to SDS-polyacrylamide gel electrophoresis. The gel was dried, stained with Coomassie Brilliant Blue and analyzed by a BAS2000 image analyzer (Fuji Photo Film, Tokyo).

Apoptosis detection assay  Terminal deoxynucleotidyl
transferase (TdT)-mediated dUTP nick end-labeling (TUNEL) assay was performed flow cytometrically using the ApopTag Direct Kit (Oncor, Gaithersburg, MD) according to the manufacturer's instructions. Cells were fixed in 1% formaldehyde solution for 15 min on ice, washed with PBS, suspended in 70% ethanol and stored at −20°C. The cells were washed with PBS and incubated in the equilibration buffer for 15 min at room temperature, followed by additional incubation in the TdT reaction buffer for 30 min at 37°C. The reaction was stopped by addition of the stop/wash buffer, and the cells were incubated in PBS containing 10 µg/ml PI and 10 µg/ml RNase A for 15 min at room temperature in the dark. Data of DNA strand breaks (fluorescein) and DNA content (PI) from 20,000 cells were subjected to bivariate plot analysis using the Multi2D program (Phoenix Flow Systems, San Diego, CA). Basal level of apoptosis was validated using the control specimen and the R1 region was set. Cells with fluorescence within the R1 region were considered apoptotic.

RESULTS

Cell growth inhibition Table I shows that UCN-01 inhibited the growth of various human cultured cell lines with 50% growth-inhibitory concentrations (IC50s) ranging from 30 to 500 nM and with 80% growth-inhibitory concentrations (IC80s) ranging from 100 to 3500 nM. Sensitivities of the cell lines to UCN-01 appeared to lack correlation with p53 status in the cell lines. In addition, status of Rb protein does not necessarily predict the sensitivities of these cell lines to UCN-01.

Cell cycle distribution To elucidate the importance of p53 as well as Rb for the effect of UCN-01 on cell cycle distribution, we selected colon carcinoma cell lines WiDr and HCT116, which harbor mutated and wild-type p53, respectively, and an osteosarcoma cell line, Saos-2, which lacks both p53 and Rb. As shown in Fig. 1A, concentration-dependent G1-phase accumulation was observed after treatment of cells with UCN-01 for 24 h, and it was accompanied by a decrease of cells in the S-phase. These results demonstrate UCN-01-induced G1-phase accumulation in tumor cell lines characterized by variousproficiencies with regard to p53 and Rb. In addition, in Saos-2 cells, sub G1-phase accumulation was induced by the drug at 1000 nM (Fig. 1A), suggesting that the drug induces apoptosis.

To examine more precisely the significance of p53 and Rb for G1-phase accumulation induced by UCN-01, we compared the effects of the drug on the cell cycle distribution of human lung fibroblast WI-38 and its SV40 transformant WI-38 VA13. As shown in Fig. 1B, G1-phase accumulation was induced after treatment with UCN-01 in WI-38, but not in WI-38 VA13 cells. These results indicate that G1-phase accumulation induced by UCN-01 might be abrogated in SV40-transfected cells in which p53 and Rb proteins are inactivated by the virus.

Previously, we reported that G1-phase accumulation induced by UCN-01 in A431 human epidermoid carcinoma cells might be associated with dephosphorylation of Rb protein and inhibition of CDK2 through induction of p21Cip1/WAF1 and p27Kip1 proteins. To elucidate the contribution of these proteins to induction of G1-phase accumulation by UCN-01, we examined the effect of the drug on expression of these proteins in the cell lines tested in Fig. 1.

Rb protein phosphorylation A recent report showed that the Rb protein acquires a deficiency in its C-terminal amino acids during the apoptotic process in cells. Therefore, we used the anti-Rb antibody recognizing its C-terminal amino acids in order to detect the dephosphorylated form of the Rb protein in western blot analysis. As shown

Table I. Growth Inhibition by UCN-01 in Human Cultured Cell Lines

| Cell line | Cell type | p53 status | Rb status | IC50 nM | IC80 nM |
|-----------|-----------|------------|-----------|---------|---------|
| T47D      | Breast carcinoma | mu          | wt         | 280     | 3100    |
| MCF-7     | Breast carcinoma | wt          | wt         | 440     | 3000    |
| Hep G2    | Hepatoma   | wt          | wt         | 380     | 1500    |
| DLD-1     | Colon carcinoma | mu          | wt         | 270     | 2200    |
| WiDr      | Colon carcinoma | mu          | wt         | 210     | 1300    |
| HCT116    | Colon carcinoma | wt          | wt         | 330     | 860     |
| 5637      | Bladder carcinoma | mu          | null      | 200     | 410     |
| Saos-2    | Osteosarcoma | null        | mu         | 160     | 390     |
| A549      | Non-small cell lung carcinoma | wt | wt         | 99      | 220     |
| WI-38     | Lung fibroblast | wt          | wt         | 40      | 180     |
| WI-38 VA13| SV40 transformant | SV40       | SV40      | 34      | 110     |

a) Determined by MTT assay as described in “Materials and Methods.”
b) Mutant.
c) Wild type.
in Fig. 2, the Rb protein was shown to be constitutively expressed and highly phosphorylated in WiDr and HCT116 cells, and it was dephosphorylated after treatment with UCN-01 in both cell lines (Fig. 2). In Saos-2 cells, the Rb protein was not detected, consistent with a previous report, and it was also not detected after treatment with UCN-01 (Fig. 2). In WI-38 cells, the Rb protein was shown to be constitutively highly phosphorylated, and was dephosphorylated dramatically during treatment with UCN-01 (Fig. 2). In contrast, in WI-38 VA13 cells, the Rb protein was shown to be constitutively partially phosphorylated, and the phosphorylation state was not altered by treatment with UCN-01 (Fig. 2). These results suggest that the Rb protein may not be required for G1-phase accumulation induced by UCN-01. However, the degree of dephosphorylation of Rb protein induced by UCN-01 in the Rb-positive cell lines appeared to parallel the intensity of G1-phase accumulation, suggesting that the Rb protein might contribute, at least in part, to this cell cycle effect in Rb-positive cells.

Fig. 1. Effect of UCN-01 on cell cycle distributions in human cultured cell lines. Cells were treated with UCN-01 for 24 h at the indicated concentrations. Cell harvest and cell cycle analysis were performed as described in “Materials and Methods.” A, WiDr, HCT116 and Saos-2 cell lines; B, WI-38 and WI-38 VA13 cell lines.
p21Cip1/WAF1 and p27Kip1 protein expressions As shown in Fig. 3A, the p21Cip1/WAF1 protein was constitutively highly expressed in the HCT116, WI-38 and WI-38 VA13 cell lines. p21Cip1/WAF1 protein levels were not significantly altered during treatment with UCN-01 at 100 and 300 nM in HCT116 cells and at 30 and 100 nM in WI-38 and WI-38 VA13 cells and they were apparently decreased during treatment with the drug at 1000 nM in HCT116 cells and at 300 nM in WI-38 and WI-38 VA13 cells. In WiDr cells, the p21Cip1/WAF1 protein was not constitutively expressed, and the level was slightly up-regulated after treatment with UCN-01 (Fig. 3A). Furthermore, p21Cip1/WAF1 protein was undetectable after treatment with or without UCN-01 in Saos-2 cells (Fig. 3A). As shown in Fig. 3B, the p27Kip1 protein was constitutively highly expressed in HCT116, WI-38 and WI-38 VA13 cell lines, and its level was slightly increased by treatment with UCN-01. In the WI-38 and WI-38 VA13 cell lines however, p27Kip1 protein levels were slightly decreased after treatment with UCN-01 at 300 nM (Fig. 3B). In the WiDr and Saos-2 cell lines, p27Kip1 protein levels were low in the absence of the drug and markedly up-regulated during treatment with UCN-01 (Fig. 3B). These results indicate that the increase of the p21Cip1/WAF1 protein was not inevitably linked to G1-phase accumulation after treatment with UCN-01, and that the increase of the p27Kip1 protein might play a role in induction of G1-phase accumulation caused by UCN-01 in Rb-functional cell lines.

CDK2 protein expression and its kinase activity As shown in Fig. 4, CDK2 protein was expressed in all cell lines tested. In WiDr, HCT116 and Saos-2 cells, the active form of CDK2 protein (phosphorylated at Thr160) was

**Table II. Summary of Effects of UCN-01 on Apoptosis, Cell Cycle and Their Regulatory Proteins in Human Cell Lines**

| Cell line | PARP | TdT | sub G1 | G1 | ppRb | p21 | p27 | CDK2-P | CDK2 act. |
|-----------|------|-----|--------|----|------|-----|-----|--------|----------|
| WiDr      | −    | NT  | −      | +  | ↓    | ↑   | ↑   | ↓      | ↓        |
| HCT116    | −    | −   | −      | +  | ↓    | ↓/↑| ↑/↓| ↓      | ↓        |
| Saos-2    | +    | +   | +      | +  | ND   | ND  | ↑   | ↓      | ↓        |
| WI-38     | −    | NT  | −      | +  | ↓    | →/↓| ↑/↓| ↓      | ↓        |
| WI-38 VA13| −    | NT  | +      | −  | →    | →/↑| ↑/↑| ↓/↑    | ↑        |

PARP, PARP cleavage into 85 kDa fragment; TdT, TdT-positive cells; sub G1, cells with DNA<2n; G1, G1-phase accumulation; ppRb, hyperphosphorylated Rb protein; p21, p21Cip1/WAF1 protein; p27, p27Kip1 protein; CDK2-P, Thr160-phosphorylated CDK2 protein; CDK2 act., cellular CDK2 kinase activity; ↑, induced; →, not induced; ↑, increased; ↓, decreased; →, not changed; NT, not tested; ND, not detected.
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reduced in a concentration-dependent manner during treatment with UCN-01 (Fig. 4). In addition, the active form of CDK2 in WI-38 cells was constitutively slightly expressed and was rendered undetectable by treatment with UCN-01 (Fig. 4). In contrast, the active form in WI-38 VA13 cells was constitutively highly expressed and was not altered by treatment with UCN-01 (Fig. 4).

As shown in Fig. 5, cellular CDK2 kinase was constitutively active in all these cell lines when they were growing. The kinase activity was reduced in a concentration-dependent manner by treatment with UCN-01 in the WiDr, HCT116 and Saos-2, UCN-01 at 0, 100, 300 and 1000 nM (lanes 1–4, respectively); WI-38 and WI-38 VA13, UCN-01 at 0, 30, 100 and 300 nM (lanes 1–4, respectively).

Induction of apoptosis Fig. 1 demonstrates the appearance of a sub G1-phase cell population during treatment with UCN-01 at 1000 nM in Saos-2 and at 100 and 300 nM in WI-38 VA13 cells, suggesting induction of apoptosis. Induction of apoptosis by UCN-01 was further assessed by other approaches such as detection of apoptosis-related proteins and DNA fragmentation. As shown in Fig. 6, PARP protein (116 kDa), the major death substrate of caspase 3, was constitutively expressed in all cell lines, and its expression level was decreased after treatment with UCN-01 for 24 h at the highest concentration in all cell lines except HCT116 cells (Fig. 6). In addition, the apoptotic degradation product of PARP (85 kDa) was detected only in Saos-2 cells (Fig. 6). Furthermore, as shown in Fig. 7, apoptotic DNA fragmentation, which was recognized by the TUNEL assay, was intensively induced in Saos-2 cells after treatment with UCN-01. In contrast, DNA fragmentation was not induced in HCT116 cells, although the sub G1-phase population was slightly increased at the highest concentration tested. These results
clearly demonstrate that UCN-01 induces apoptosis in Rb-defective Saos-2 cells and WI-38 VA13 cells.

DISCUSSION

We reported previously that G1-phase accumulation induced by UCN-01 might be associated with CDK2 inhibition mediated through its dephosphorylation at Thr160 and induction of CDK inhibitor proteins such as p21\(^{C^\text{i}}\)/WAF1 and p27\(^{K^\text{ip}}\) in A431 cells.\(^{17}\) In this study, UCN-01-induced G1-phase accumulation was demonstrated to be correlated with the reduction of cellular CDK2 kinase activity and the down-regulation of its active Thr160-phosphorylated form in several human tumor cell lines (Fig. 4, Table II). One possible mechanism of down-regulation of active CDK2 by UCN-01 is inhibition of CAK. The direct inhibitory activity of UCN-01 against CAK remains to be determined. It is possible that UCN-01 inhibits CAK indirectly. One may speculate that CDK inhibitor proteins induced by UCN-01 bind to the inactive form of CDK2 and prevent its phosphorylation by CAK.\(^{34}\) We cannot exclude the alternative possibility that UCN-01 promotes the degradation of Thr160-phosphorylated CDK2 protein by an as yet unknown mechanism.

Our data suggest that the contribution of the p27\(^{K^\text{ip}}\) protein to the CDK2 inhibition by treatment with UCN-01 may be significant in the WiDr and Saos-2 cell lines (Fig. 3). Cellular p27\(^{K^\text{ip}}\) protein levels are known to be mainly regulated by proteolysis via a ubiquitin-proteasome pathway.\(^{35}\) We previously examined the effect of UCN-01 on p27\(^{K^\text{ip}}\) mRNA level in A431 cells, in which p27\(^{K^\text{ip}}\) protein levels were up-regulated by the drug near the IC\(_{50}\).\(^{17}\) The result showed that the mRNA level was not altered by treatment with UCN-01 (data not shown). Therefore, the mechanism of the increase of cellular p27\(^{K^\text{ip}}\) protein level by UCN-01 remains unclear. Recent reports suggest that the subcellular location of the p27\(^{K^\text{ip}}\) protein is important for its function as a CDK inhibitor.\(^{36, 37}\) This implies that treatment of cells with UCN-01 may change the subcellular location of p27\(^{K^\text{ip}}\) protein and thus convert the protein from a non-functional to a functional CDK inhibitor.

A previous report has shown that UCN-01 activates CDK2 and CDK1 kinases in Jurkat human leukemic-T cells.\(^{18}\) Very recently, we observed that UCN-01 induced apoptosis rather than G1-phase accumulation in A549 cells after 24 h exposure at concentrations higher than the IC\(_{50}\), which was accompanied by maintenance of, but not reduction in, the cellular CDK2 kinase activity.\(^{38}\) In this study, the apoptosis induced by UCN-01 might correlate with the incomplete reduction of cellular CDK2 kinase activity in WI-38 VA13 cells (Fig. 5, Table II). These data imply the possibility that the remaining CDK2 kinase activity after treatment with UCN-01 may allow cells to progress through the G1/S-phase boundary and to undergo apoptosis. A recent study reported that p27\(^{K^\text{ip}}\) and p21\(^{C^\text{i}}\)/WAF1 proteins were cleaved by CPP32-like caspase,\(^{39}\) hinting at the possibility that the decrease of expression of CDK inhibitor proteins after treatment with high concentrations of UCN-01 might be the corollary of cleavage by activated caspase (Fig. 3). These cleaved CDK inhibitor proteins may correlate with the incomplete reduction of cellular CDK2 kinase activity after treatment with UCN-01.

We demonstrate here that UCN-01 induced G1-phase accumulation in four human cell lines harboring p53 and Rb in various amounts and phosphorylation states, suggesting that these proteins might not be directly involved
in the induction of G1-phase accumulation by the drug. This conclusion is supported by the observation that G1-phase accumulation was also induced intensively in MCF-7 (p53 wild type/Rb wild type) and somewhat in 5637 (p53 mutated/Rb null) cells (data not shown). However, a good correlation was observed between the intensity of dephosphorylation of Rb protein and G1-phase accumulation induced by UCN-01 in the WiDr and HCT116 cell lines (Fig. 2, Table II). In addition, induction of both G1-phase accumulation and dephosphorylation of the Rb protein by UCN-01 was much more intense in WI-38 cells than in WI-38 VA13 cells (Figs. 2 and 3, Table II). These observations suggest that Rb or its related proteins such as p107 and p130, which are targeted by SV40 large T-antigen, may, at least in part, contribute to G1-phase accumulation induced by UCN-01. This suggests that cells deficient in G1-checkpoint function (p53 mutated/Rb null) cells (data not shown). However, a unique G1-checkpoint response in the cells exposed to UCN-01 seems important, because a clear mechanistic understanding might help identification of determinants of sensitivity of individual tumors to UCN-01 in the clinical context.

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