Cyclin B and E2F-1 Expression in Prostate Carcinoma Cells Treated with the Novel Retinoid CD437 Are Regulated by the Ubiquitin-mediated Pathway

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ABSTRACT

E2F-1 and cyclin B are important regulators of the cell cycle, and their expression and degradation are tightly regulated. Proteolysis of both molecules is mediated by the ubiquitin degradation pathway involving the activation of specific E3 ubiquitin ligases. Treatment of prostate carcinoma cells with the novel retinoid 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437/AHPN) results in the enhanced expression of E2F-1 and rapid degradation of cyclin B in the absence of the modulation of mRNA levels; this is accompanied by the S phase arrest of the cells and subsequent apoptosis. The elevated level of E2F-1 is because of the enhanced stability of the molecule, as indicated by pulse-labeling studies, demonstrating a prolonged half-life. The enhanced E2F-1 stability is associated with the concomitant acetylation of E2F-1, the dissociation of E2F-1 from the E2F-1 E3 ligase p45SKP2, and decreased E2F-1 ubiquitination, suggesting CD437 inhibition of E-3 E2F-1 ligase activity. Exposure of the cells to CD437 also results in the enhanced association of the cyclin B E3 ligase APC with cyclin B and the rapid proteolysis of cyclin B. The CD437-enhanced proteolysis of cyclin B is blocked in the presence of the ubiquitin proteolysis inhibitor N-acetyl-leu-leu-norleucinal. Thus, CD437 modulates the expression of E2F-1 and cyclin B through the simultaneous stimulation and inhibition of the cyclin B and E2F-1 E3 ligases, respectively.

INTRODUCTION

The ability of retinoids to modulate the growth of both normal and transformed cells has been well described (1). Retinoid-mediated alterations of gene expression through binding to their nuclear receptor, i.e., RARs and RXRs, are felt to be responsible for the biological actions of these compounds (2). The retinoid/receptor nuclear receptor complexes bind in turn to specific motifs, RAR and RXR elements located in the regulatory regions of genes, and, thus, modify their expression (3). Retinoid activation of these receptors in turn is known to play an important role in retinoid-mediated growth inhibition of different carcinoma cell types. Prostate carcinoma cells display resistance to the antiproliferative effects of retinoids (4, 5). The reason for this resistance is not clear, but it has been speculated that it may be secondary to the loss of RARβ nuclear receptor expression (4). We have described previously a novel retinoid CD437/AHPN, which induces S phase arrest and apoptosis in LNCaP and PC-3 prostate carcinoma cells (6).

The induction of S phase cell cycle arrest and apoptosis has been found to be often associated with the inappropriate expression of the nuclear transcription factor E2F-1 (7–9). The expression and biological activity of the E2F family of transcriptional activators are regulated tightly throughout the cell cycle (10). E2F-1 is tightly bound by the unphosphorylated Rb protein during the early and midportions of the G1 phase of the cell cycle. Phosphorylation of Rb by cyclin D complexed to Cdk 4/cdk 2 or by cyclin E/cdk2 results in the release of E2F-1 and subsequent binding of E2F-1/DP-1 heterodimer to the E2F-1 consensus sequences located in the promoters of a number of genes (11–14). This allows for the activation of these genes and progression of cells through the G1 checkpoint and into the S phase of the cell cycle (9, 15). In addition, the E2F-1 promoter also contains an E2F-1 consensus sequence allowing for enhanced E2F-1 gene transcription and production (16). Because DP-1 is always in excess, this allows for an even greater formation of DP-1/E2F-1 heterodimers and gene transcription (10). Inactivation of the E2F-1/DP-1 heterodimer through phosphorylation of DP-1 by cyclin A/cdk 2 with loss of E2F-1/DP-1 binding to its consensus sequence is also necessary for normal progression through S phase (17, 18). Failure to inhibit the E2F-1/DP-1 heterodimer binding has been found to result in S phase cell cycle arrest with subsequent apoptosis (17–19). A number of studies has now demonstrated that E2F-1 expression is regulated by the ubiquitin proteasome pathway involving a specific motif located in the COOH terminus of the E2F-1 molecule (20–22).

Cyclin B1 also plays an important role in cell cycle progression. Cyclin B1 expression is minimal at the initiation of S phase and peaks at the G2-M border; this peak of cyclin B1 activity is required for cells to enter mitosis (23). Regulation of cyclin B1 expression is complex and is found to occur at the transcriptional and post-transcriptional, as well as post-translational levels (23–28). The cyclin B1 promoter displays cell cycle regulation with minimal activity at G0 and maximal activity at the G2 phase of the cell cycle (23). Activation of p53 can result in the inhibition of cyclin B1 promoter activity at the G2-M phase of the cell cycle resulting in G2-M arrest (29). Cyclin B1 mRNA levels are also regulated through the presence of cis-stability motifs and the corresponding trans-factors (24). Expression of these trans-factors appears to be cell cycle regulated such that the stability of cyclin B1 mRNA is minimal at G1 but peaks at G2-M (24). In addition, cyclin B1 is degraded by the ubiquitin pathway through the activation of the APC (27, 28); activation of the APC through specific phosphorylation of its components and the synthesis or activation of cyclin B1-directing components with the subsequent degradation of cyclin B appears to be necessary for the exit from mitosis (27).

We have found that exposure of the prostate carcinoma cell lines LNCaP, DU145, and PC-3 to CD437 results in the rapid increase in E2F-1 levels accompanied by a concurrent decrease in cyclin B levels. CD437 modulation of the E2F-1 and cyclin B levels occurs through the ubiquitin pathway with the simultaneous inhibition of E2F-1 degradation and activation of cyclin B degradation.

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3 The abbreviations used are: RAR, retinoic acid receptor; RXR, retinoid X receptor; 7-ADD, 7-aminoadamantyl-2-naphthalene carboxylic acid; CD347/AHPN, 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid; BrdUrd, 5-bromo-2’-deoxyuridine; Rb, retinoblastoma; cdk, cyclin dependent kinase; BDIS, Becton Dickinson Immunocytometry Systems; APC, anaphase-promoting complex; LMM, N-acetyl-leu-leu-norleucinal.

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 MATERIALS AND METHODS 

**Chemicals and Antibodies.** AHPN/CD437 was synthesized as described previously (30), dissolved in DMSO to a concentration 5 mM, and stored at −80°C. DMEM, DMEM-F12 medium, FBS, and dialyzed FBS were purchased from Life Technologies, Inc. (Grand Island, NY). Anticyclin B1, anti-E2F-1, anti-CDC20, anti-p45 SRKp2, and antibuiquitin antibodies were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA); anti-Cdc27 antibody was obtained from Transduction Laboratories (Lexington, KY). The acetylated lysine antibody was obtained from Cell Signaling (Beverly, MA). The ICDH1 antibody has been described previously (31, 32). The proteasome inhibitor LLnL, horseradish peroxidase-conjugated secondary antibody, and histone H1 were purchased from Sigma (St. Louis, MO).

**Cultures and Cell Cycle Analysis.** The human prostate carcinoma cell lines DU145, LNCaP, and PC-3 were maintained in DMEM-F12 medium supplemented with 5% FBS, at 37°C in a humidified incubator with 5% CO2. For flow cytometry analysis, DU145 cells were treated with 1 µM CD437 for 24 and 48 h, and the cells were pulse labeled with BrdUrd, a thymidine analogue, for 2 h using a BrdUrd flow kit (PharMingen Laboratories, San Diego, CA). BrdUrd incorporation was detected with a FITC-conjugated anti-BrdUrd antibody, and DNA content was labeled with 7-AAD.

Flow cytometry was performed on a FACSscan flow cytometer (BDIS, San Jose, CA) equipped with an argon ion laser tuned to 15 mW at 488 nm for fluorescence excitation and light scattering, controlled by a Power Macintosh G3 (Apple Computer, Cupertino, CA) running CELLQuest software (BDIS). FITC fluorescence was collected in the FL1 detector using a 530/30 nm bandpass filter, and 7-AAD fluorescence was reflected with a 560 nm long pass filter.

**Western Blot and Immunoprecipitation.** Cells were lysed with lysis buffer [50 mM Tris (pH 7.5), 100 mM NaCl, 1 mM EDTA, 0.5% NP40, 0.5% Triton X-100, 2.5 mM sodium orthovanadate, 10 µM protease inhibitor cocktail (Sigma), and 1 mM phenylmethylsulfonyl fluoride], and the protein concentration was determined using the Bio-Rad assay method (Bio-Rad Laboratories, Hercules, CA). Proteins (75 µg) were fractionated using 12% SDS-PAGE and transferred onto nitrocellulose membranes. The membranes were blocked with 5% nonfat dried milk in either PBS or Tris-buffered saline buffer containing 0.1% Tween 20 and then incubated with the appropriate primary antibody and the procedure of Fang et al. (32). Proteins were eluted with Laemmli sample buffer and fractionated using 12% SDS-PAGE, and Western blots were performed.

**Cyclin B Kinase Assay.** Cells were lysed with lysis buffer containing 5 µg/ml aprotinin, 5 µg/ml pepstatin A, and 5 µg/ml leupeptin. Proteins (500 µg) were immunoprecipitated with anticyclin B1 polyclonal antibody, and the immunoprecipitates were washed three times with TT buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.5% Tween 20] and twice with lysis buffer. Coimmunoprecipitation studies were performed using antibody and the procedure of Fang et al. (32). Proteins were eluted with Laemmli sample buffer and fractionated on 12% SDS-PAGE. The gels were fixed, and the labeled bands were visualized by autoradiography.

**Pulse Chase Experiment.** DU145 cells were grown in DMEM medium supplemented with 5% FBS and then incubated in DMEM medium without methionine and cysteine and supplemented with 5% dialyzed FBS for 1 h. Cells were labeled with [L-[35S] methionine (100 µCi) and L-[35S] cysteine (1000 Ci/mmol; Amersham Pharmacia Biotech) for 2 h and then incubated in DMEM, supplemented with L-methionine and L-cysteine both at 5 µg/ml, 10% FBS, and in the presence and absence of 1 µM CD437. Cells were trypsinized and lysed with lysis buffer at 0, 2, 4, and 6 h after the addition of unlabelled L-methionine and L-cysteine. Protein extracts (1000 µg) were immunoprecipitated with anti-E2F-1 antibody and resolved on 12% SDS-PAGE. The gel was then fixed and exposed to film, and the bands were quantified using laser densitometry.

**Northern Blots.** RNA was extracted, and Northern blots were performed as we have described previously (36).

## RESULTS

**CD437 Induces S Phase Arrest and Apoptosis in DU145 Cells.** We have found previously that exposure of PC-3 and LNCaP cells to CD437 results in S phase cell cycle arrest followed by apoptosis (6). We determined whether similar results would be found with the DU145 prostate carcinoma cell line. Exposure of DU145 cells to 1 µM CD437 results in the progressive increase in cells accumulating in S phase of the cell cycle (Fig. 1, A–D); these cells arrest in S phase after 24- (85% in S phase) and 48-h (83% in S phase) exposure to CD437 as opposed to exposure to vehicle only, which results in only 32 and 35% in S phase at 24 and 48 h. Although these cells accumulate in S phase, this is accompanied by a progressive decrease in BrdUrd labeling. This decrease in BrdUrd labeling is secondary to the rapid onset of apoptosis in these cells with 20 and 60% undergoing apoptosis by 24 and 48 h, respectively (Fig. 1, E and F). There is a progressive shift in the cells arrested in S phase to the G1-G2 phase (Fig. 1D compared with Fig. 1C); this represents the progressive accumulation of apoptotic cells. The S phase arrest noted in the prostate carcinoma cell lines was in distinct contrast to breast carcinoma cells in which exposure to CD437 resulted in a G1 phase arrest (6, 36).

**Exposure to CD437 Results in Elevated E2F-1 Levels.** Inappropriate elevation of E2F-1 levels has been demonstrated to induce S phase arrest and apoptosis in several cell lines (17–19). Therefore, we examined E2F-1 levels in DU145, LNCaP, and PC-3 after exposure to CD437. Exposure of E2F-1 levels was noted within 6 h of CD437 addition to the cells and persisted for >48 h (Fig. 2A). Exposure to CD437 resulted in a 4- and 6-fold, 2- and 3-fold, and 2- and 2.5-fold increase in E2F-1 levels in DU145, LNCaP, and PC-3 cells, respectively, at 6 and 24 h. E2F-1 mRNA levels did not increase after the addition of CD437 in any of the cell lines (Fig. 2B), suggesting that the CD437-mediated increase in E2F-1 levels may occur through enhanced stability of the protein. Pulse chase experiments were performed in which E2F-1 was labeled with [35S] methionine and [35S] methionine, and E2F-1 degradation was assessed (Fig. 3, A and B). Exposure to CD437 results in a significant increase in the half-life of E2F-1, confirming increased stability of E2F-1 in the presence of CD437 (Fig. 3, A and B).
E2F-1 and the ubiquitin-protein ligase SCF SKP2 (p45 SKP2) results in marked reduction in the ubiquitination of E2F-1 and the subsequent stabilization of E2F-1 levels (37). To determine whether the addition of CD437 to the prostate carcinoma cells results in a decrease in the association between E2F-1 and p45 SKP2, cells were grown in the absence and presence of CD437, p45 SKP2 immunoprecipitated, and the associated E2F-1 levels were determined by Western blot (Fig. 4).

Exposure to CD437 resulted in a significant decrease in the E2F-1-associated p45 SKP2 (Fig. 4). Interestingly, a marked decrease in the p45 SKP2 E2F-1-associated levels was noted at 6 h, followed by a mild increase at 24 and 48 h; the p45 SKP2 E2F-1-associated levels noted at 24 and 48 h were still significantly below those noted in the vehicle-treated cells. The decreased E2F-1-bound p45 SKP2 was not because of a decrease in p45 SKP2 levels because no decrease in total p45 SKP2 was noted after exposure to CD437 (Fig. 4). The specificity of the interaction between E2F-1 and p45 SKP2 was indicated by the fact that no E2F-1 is detected if a non-p45 SKP2 antibody is used to perform the immunoprecipitations.

CD437 Enhances E2F-1 Acetylation. CD437 treatment of prostate carcinoma cells resulted in marked reduction in the ubiquitination of E2F-1 and the subsequent stabilization of E2F-1 levels (37). To determine whether the addition of CD437 to the prostate carcinoma cells results in a decrease in the association between E2F-1 and p45 SKP2, cells were grown in the absence and presence of CD437, p45 SKP2 immunoprecipitated, and the associated E2F-1 levels were determined by Western blot (Fig. 4).

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CD437 Enhances E2F-1 Acetylation. CD437 treatment of prostate carcinoma cells followed by 12% SDS-PAGE suggested the presence of two E2F-1 bands (Fig. 3). To further demonstrate the presence of these two bands and assess whether CD437 modulated their expression, DU145 cells were treated and exposed to CD437 in the presence and absence of cycloheximide, and the fractionation of proteins was performed using 10% SDS-PAGE, allowing for separation of the bands. Exposure to CD437 resulted in the increase in total E2F-1 and a faster migrating E2F-1 species (Fig. 5A, Lane 2). Exposure to CD437 and cycloheximide decreased the expression of the slower migrating E2F-1 band, but there was no modulation of the faster migrating band level. This result suggested that incubation with CD437 resulted in the post-translational modification of E2F-1.

Recent studies have demonstrated that modification of E2F-1 either
CD437 modulation of E2F-1 and cyclin B expression

Fig. 5. CD437 increased acetylated E2F-1 levels. DU145 cells (1 × 10^6 cells/ml) were exposed to vehicle or 1 μM CD437 in the presence and absence of cycloheximide (20 μg/ml). In A, cells were harvested at 6 h, and Western blots were performed using 10% SDS-PAGE, as described in “Materials and Methods.” Lane 1, cells treated with vehicle alone; Lane 2, cells treated with CD437; Lane 3, cells treated with cycloheximide; Lane 4, cells treated with cycloheximide and CD437. The results depicted are representative of two independent experiments. Equal loading was assessed using actin levels. B, CD437-enhanced acetylation of E2F-1. Prostate carcinoma cells were exposed to 1 μM CD437 for 24 h. The cells were harvested and lysed, and immunoprecipitation with E2F-1 antibody was performed as described in “Materials and Methods.” Western blots were performed using antiacetylated lysine antibody as described in “Materials and Methods.”

CD437-mediated increase in E2F-1 levels results in enhanced E2F-1 binding to the E2F-1 consensus sequence. We have found that CD437-mediated increase in E2F-1 levels is associated with S phase arrest and apoptosis in normal mammary epithelial cells (35). Previous studies have demonstrated that enhanced binding of E2F-1 to its consensus sequence is associated with E2F-1-mediated apoptosis (19). Therefore, we determined whether the CD437-mediated increase in E2F-1 levels is associated with enhanced E2F-1 binding to its consensus sequence. The appearance of an additional E2F-1 complex binding to the E2F-1 consensus sequence after exposure to CD437 is demonstrated in Fig. 7 (see arrow). This binding indeed represented E2F-1 because it was only competed by an excess of unlabelled E2F-1 consensus sequence and not by a 1000-fold excess of unlabeled nonspecific probe (double-stranded p21WAF1/CIP1 3′ untranslated region of 108 bp; Fig. 7A) and supershift of the binding activity in the presence of E2F-1 antibodies (arrow, Fig. 7B).

CD437 enhances cyclin B proteolysis and cyclin B ubiquitin levels. Although CD437 treatment of the prostate carcinoma cell lines resulted in an elevation of E2F-1, a marked decrease in cyclin B levels (Fig. 8A) and cyclin B kinase activity (Fig. 8B) in the three prostate carcinoma cell lines was also noted. Cyclin B mRNA levels remain unchanged. This result suggested that whereas CD437 inhib-
CD437 MODULATION OF E2F-1 AND CYCLIN B EXPRESSION

Fig. 7. CD437-mediated increase in E2F-1 levels is associated with increased binding of E2F-1 to its consensus sequence. Nuclear extracts were prepared from DU145 cells after exposure to vehicle or 1 μM CD437 for 24 h; labeling of probe and gel shifts were performed as described in “Materials and Methods.” A, Lane 1, probe alone; Lane 2, probe and nuclear protein extracts from untreated cells; Lane 3, probe nuclear protein extracts from untreated cells and 1000-fold excess of cold, unlabeled probe fragment DNA; Lane 4, probe nuclear protein extract from untreated cells and 1000-fold excess of cold, unlabeled, and double-stranded p21WAF1/CIP1 3′-untranslated region of 108 bp; Lane 5, probe nuclear protein extracts from CD437-treated cells; Lane 6, probe nuclear protein extracts from CD437-treated cells and 1000-fold excess of cold, unlabeled probe fragment DNA; Lane 7, probe nuclear protein extracts from CD437-treated cells and 1000-fold excess of cold, unlabeled, and double-stranded p21WAF1/CIP1 3′-untranslated region of 108 bp. B, Lane 1, probe alone; Lane 2, probe nuclear protein extracts from untreated cells; Lane 3, probe nuclear protein extracts from untreated cells and E2F-1 antibody; Lane 4, probe nuclear protein extract from CD437-treated cells; Lane 5, probe nuclear protein extracts from CD437-treated cells and E2F-1 antibody. The results are representative of two independent experiments. Arrow, the appearance of the new E2F-1 complex after CD437 exposure.

Fig. 8. The addition of CD437 to prostate carcinoma cells results in decreased cyclin B levels (A) and decreased cyclin B kinase activity (B). Prostate carcinoma cells were exposed to 1 μM CD437 for various periods of times. Cells were harvested, and Western blot for cyclin B levels (A) and cyclin B kinase activities (B) were determined as described in “Materials and Methods.”

Fig. 9. LLnL blocks CD437-mediated decrease in cyclin B levels. Prostate carcinoma cells were grown in the presence of vehicle alone, 1 μM CD437, 1 μM CD437 in the presence of various concentrations of LLnL, and various concentrations of LLnL alone. The cells were harvested after 24 h, and cyclin B levels were assessed by Western blot. DU145 cells: Lane 1, control; Lane 2, 1 μM CD437; Lane 3, 2.5 μM LLnL; Lane 4, 2.5 μM LLnL and 1 μM CD437; Lane 5, 5 μM LLnL; Lane 6, 5 μM LLnL and 1 μM CD437. PC-3 cells: Lane 1, control; Lane 2, 1 μM CD437; Lane 3, 2.5 μM LLnL; Lane 4, 2.5 μM LLnL and 1 μM CD437; Lane 5, 10 μM LLnL; Lane 6, 10 μM LLnL and 1 μM CD437.

DISCUSSION

E2F-1 and cyclin B are important regulatory molecules, which play essential roles in the progression of cells along the cell cycle. The ubiquitin pathway-mediated degradation of these proteins is tightly regulated. In this study, we have demonstrated that exposure of the prostate carcinoma cell lines DU145, LNCaP, and PC-3 to the novel retinoid CD437 results in the enhanced stability of E2F-1 with the concomitant instability of cyclin B through CD437 modulation of the ubiquitin-mediated pathway; these modulations of E2F-1 and cyclin B levels are associated with the onset of S phase cell cycle arrest and subsequent apoptosis of these cells.

The cells were grown in the absence or presence of the ubiquitin proteolysis inhibitor LLnL and in the absence and presence of CD437. If the CD437-induced decrease in cyclin B levels is modulated through the ubiquitin pathway, then inhibition of this pathway should block CD437-mediated decrease in cyclin B levels. Incubation with the proteolysis inhibitor LLnL inhibited the CD437-mediated decrease in cyclin B levels, demonstrating that CD437 enhanced ubiquitin pathway-mediated proteolysis of cyclin B (Fig. 9). Activation of the E-3 ubiquitin-protein ligase APC has been shown to play an essential role in cyclin B degradation (27, 40). To document the potential APC involvement in the CD437-mediated decrease in cyclin B levels, prostate carcinoma cells were exposed to CD437, cyclin B immunoprecipitates were fractionated on SDS-PAGE, and Western blots performed with anti-Cdc 27 antibody; Cdc27 is an essential component of APC (38). An increased association of Cdc 27 with cyclin B was noted in the prostate carcinoma cells after exposure of the cells to CD437 (Fig. 10A). Targeting of activated APC to cyclin B requires the activation of hCDH1 and its binding to APC (31, 41–46). Therefore, we investigated whether exposure to CD437 results in enhanced binding of hCDH1 to APC; increased binding of hCDH1 to Cdc27 was indeed found after exposure of the cells to CD437, whereas no modulation of cellular hCDH1 levels was detected (Fig. 10B). The specificity of the interaction between hCDH1 and Cdc27 was indicated by the lack of a Cdc 27 band if a non-hCDH1 antibody was used for the immunoprecipitations. If CD437 exposure results in the increased association of cyclin B with APC ligase, then enhanced cyclin B ubiquitin levels should be noted after the exposure of the cells to CD437. Increased cyclin B ubiquitin levels were indeed noted after exposure of DU145, LNCaP, and PC3 cells to CD437 (Fig. 10C).
The activation of the cdc2/cyclin B complex serves as the trigger for entry into mitosis (47). Cyclin B while synthesized in the cytoplasm shuttles between the nucleus and the cytoplasm; phosphorylation of its nuclear translocation signal results in its rapid accumulation in the nucleus at the time of entry into mitosis (48, 49). Full activation of the cdc2/cyclin B complex, which occurs both in the cytoplasm and nucleus, requires the dephosphorylation of cdc2 at Thr 14 and Tyr 15 by the cdc25 family of protein phosphatases (47). Cyclin B, while synthesized in the cytoplasm, requires the dephosphorylation of its nuclear translocation signal results in its rapid accumulation in the nucleus at the time of entry into mitosis (48, 49).

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CD437 is a novel retinoid that, although binding to RARy, has been shown by a number of investigators to mediate apoptosis through a pathway not involving the RARs or RXRs (60–62); thus, CD437 may exert its effect in the prostate carcinoma cells through a unique pathway not involving these retinoid nuclear receptors. The contention that the RARs are not involved in CD437 modulation of E2F-1 and cyclin B levels and its induction of apoptosis is supported by the observation that trans-retinoic acid, which is a much better activator of the RARs than CD437, neither inhibits the growth nor induces apoptosis in these cell lines, even at concentrations of 10 μM (5, 63). Perhaps more importantly, exposure of these cells to CD437 results in their death; whether CD437 or its analogs will prove to demonstrate similar activity in vivo against prostate carcinoma is now under investigation.

In this study, we have made the unique observation that exposure to the novel retinoid CD437 results in inhibition of E2F-1 proteolysis but stimulation of cyclin B proteolysis, both through the ubiquitin-mediated pathway. The exact mechanisms by which CD437 exerts these paradoxical effects on the E3 ligases involved are now being examined.

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