Cyclin D1 Degradation Is Sufficient to Induce G₁ Cell Cycle Arrest despite Constitutive Expression of Cyclin E2 in Ovarian Cancer Cells

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Abstract

D- and E-type cyclins mediate G₁-S phase cell cycle progression through activation of specific cyclin-dependent kinases (cdk) that phosphorylate the retinoblastoma protein (pRb), thereby alleviating repression of E2F-DP transactivation of S-phase genes. Cyclin D1 is often overexpressed in a variety of cancers and is associated with tumorigenesis and metastasis. Loss of cyclin D can cause G₁ arrest in some cells, but in other cellular contexts, the downstream cyclin E protein can substitute for cyclin D and facilitate G₁-S progression. The objective of this study was to determine if a flexible heteroarotinoid anticancer compound, SHetA2, regulates cell cycle proteins and cell cycle progression in ovarian cancer cells. SHetA2 induced cyclin D1 phosphorylation, ubiquitination, and proteosomal degradation, causing G₁ arrest in ovarian cancer cells despite continued cyclin E2 expression and independently of p53 and glycogen synthase kinase-3 (GSK-3). Cyclin D1 loss inhibited pRb S780 phosphorylation by cyclin D1-cdk4/6 and released p21 from cyclin D1-cdk4/6-p21 protein complexes to form cyclin E2-cdk2-p21 complexes, which repressed phosphorylation of pRb S612 by cyclin E2-cdk2 and ultimately E2F-DP transcripational activity. G₁ arrest was prevented by overexpression or preventing degradation of cyclin D1 but not by restoration of pRb S612 phosphorylation through p21 knockdown. In conclusion, we show that loss of cyclin D1 in ovarian cancer cells treated with SHetA2 is sufficient to induce G₁ cell cycle arrest and this strategy is not impeded by the presence of cyclin E2. Therefore, cyclin D1 is a sufficient therapeutic target in ovarian cancer cells. [Cancer Res 2009;69(16):6565–72]

Introduction

Ovarian cancer is the most lethal of gynecologic malignancies with more than 70% of cases diagnosed at an advanced stage that has only a 30% average 5-year survival rate. Unlike other cancers, the molecular mechanisms involved in ovarian cancer etiology are poorly understood, mainly due to difficulties in detecting and obtaining early-stage ovarian cancer lesions for study (1–3). Thus, there is a great need for low-toxicity drugs that can prevent and treat ovarian cancer.

All cancers are characterized by abnormalities in apoptosis and differentiation and altered cell proliferation (4). Cancer cells often have a selective growth advantage due to deregulation of cell cycle proteins, causing aberrant growth signaling that drives tumor development (1, 5). Exit of cells from quiescence and cell cycle progression is induced by sequential activation of cyclin-dependent kinases (cdk) by cyclins. Once the cell progresses through late G₁ into the S phase, it is irrevocably committed to DNA replication and cell division (6). Deregulation of G₁ to S-phase transition is implicated in the pathogenesis of most human cancers, including ovarian cancer (7). D- and E-type cyclins act as positive regulators of this critical transition. Cyclin D expression is the converging point at which diverse mitogenic and transformation signaling cascades integrate to mediate engagement of the cell cycle machinery (8). Three cyclin D isoforms (D1, D2, and D3) are differentially expressed in various tissues (9). Of these, cyclin D1 overexpression is most frequently associated with human cancer (10). Cyclin D1 is rarely mutated, but its overexpression confers a selective growth advantage and hence acts as a driver of neoplastic growth in various cancers (11). In epithelial ovarian cancer, overexpression of cyclin D1 has been associated with decreased survival in patients (12). An estimated 26% of sporadic epithelial ovarian cancers overexpress cyclin D1 (13). Proteosomal degradation has emerged as an important regulator of cyclin D1 levels in cancer cells (14). In addition to cyclin D, both human E-type cyclins (E/E1 and E2) are also involved in G₁- to S-phase progression and in some cases can compensate for cyclin D knockout (15–17). Overexpression of either E-type cyclin has been associated with cell proliferation and metastasis (18). The activities of both G₁ cyclins D and E converge on the retinoblastoma protein (pRb).

Unphosphorylated pRb binds to E2F-DP transcription factor heterodimers, thus masking their transactivation domain (6). In this state, pRb also recruits histone deacetylases (HDAC), SWI/SNF, and histone methylases, which all serve to maintain the repressed state of chromatin located at E2F-responsive genes required for cell cycle progression (6, 19). In healthy cells, increased cyclin D levels in response to mitogenic stimuli lead to formation of active cyclin D-cdk4/6 complexes that hypophosphorylate pRb and disrupt its association with HDAC at E2F-DP binding sites. This results in partial activation of E2F-DP, allowing transcription from the cyclin E promoter only. Ongoing pRb-SWI/SNF interactions on other E2F-DP promoters maintains E2F-DP in the repressed state (20). The newly synthesized cyclin E binds and activates cdk2, causing further pRb hyperphosphorylation resulting in additional weakening of pRb affinity for E2F-DP. The released E2F-DP transcription factor complex is now free to drive transcription of S-phase genes, hence facilitating cell cycle progression (6, 20). Disruption of the pRb pathway in cancer is not surprising given its complex control of cell cycle progression (21).
Flexible heteroarotinoids (Flex-Het) are small molecules that offer promise in ovarian cancer because they induce differentiation, apoptosis, and growth inhibition without evidence of toxicity (22–27). The lead Flex-Het, SHetA2, is currently in preclinical development and was chosen because it exhibited the greatest efficacy in inducing apoptosis in multiple cancer cell lines without killing the normal cells. The mechanism of this differential apoptosis occurs through direct effects on mitochondria and Bcl-2 family proteins (22, 24). Because apoptosis and the cell cycle are closely coupled, most compounds that are cytotoxic are also cytostatic (28). The objective of this study was to determine if alterations in cell cycle proteins and cell cycle progression contribute to the mechanism of growth inhibition induced by SHetA2 in ovarian cancer cell lines.

In this study, we report that targeting cyclin D1 for proteasomal degradation is sufficient to induce G1 cell cycle arrest in ovarian cancer cell lines. The downstream consequences on pRb phosphorylation, E2F activity, and cyclin A expression could not be compensated for by continued expression of endogenous cyclin E2 or by relief of p21 repression of cyclin E2-cdk2 activity in ovarian cancer cell lines.
cancer cell lines. Thus, cyclin D1 is a sufficient target for inducing G1 arrest of ovarian cancer cells in chemoprevention and therapeutic strategies for ovarian cancer.

Materials and Methods

Cell lines. Ovarian cancer cell lines SK-OV-3 and Caov-3 (American Type Culture Collection) were maintained in McCoy’s 5A and DMEM (with t-glutamine) media, respectively. A2780 cells were cultured in RPMI 1640 with t-glutamine (supplemented with 1 mmol/L sodium pyruvate and 1 mmol/L HEPES buffer). All media were supplemented with 10% fetal bovine serum and antibiotic/antimycotic. SHetA2 was synthesized by K. Darrell Berlin (Oklahoma State University, Stillwater, OK; ref. 25), dissolved in DMSO, and manipulated under reduced lighting conditions. All cells were treated for 24 h with 5 μM SHetA2 or DMSO unless stated otherwise.

Cell cycle analysis. Cells were seeded onto six-well plates (5 × 10⁵ per well). After treatment, cells were harvested and fixed in 70% ethanol and stored overnight at 4°C. For analysis, 1 mL of freshly prepared PBS staining solution [200 μg/mL RNase A, 20 mg/mL propidium iodide (PI), and 0.1% Triton X-100] was added to the cells. DNA content was analyzed the same day by flow cytometry (FACSCalibur from Becton Dickinson). ModFit Cell Cycle Analysis software was used to determine the cell cycle phase.

Total cell number determination and apoptosis assay. Cells were plated in six-well plates (5 × 10⁵ per well) overnight before treatment. After treatment, the medium and PBS used to wash the cells were harvested to collect any detached cells and pooled with attached cells after trypsinization. The total number of cells was determined with a Beckman Coulter counter.

For the apoptosis assay, cells were pelleted and resuspended in Annexin V-FITC-PI stain (100 μL Annexin binding buffer, 5 μL Annexin V-FITC conjugate, and 2 μL of 100 μg/mL PI; Invitrogen) for 10 min at room temperature. An additional 400 μL of the Annexin binding buffer was then added and the samples were run through a FACSCalibur. Summit for MoFlo Acquisition and Sort Control Software (Cytomation, Inc.) were used to quantify apoptosis.

Transient expression of cyclin D1. Cells were transfected with 5 μg human pcDNA3 cyclin D1 T286 mutant (Mt) and pcDNA3 cyclin D1 wild-type (Wt) using Metafectene Pro transfection reagent (Biontex) according to the manufacturer's protocol. Plasmids were a kind gift from Dr. Osamu Tetsu (University of California San Francisco, San Francisco, CA) and were previously described (29). After 24 h, the cells were replated and cultured for a further 24 h before treatment.

Immunoprecipitation. Cells transfected with 10 μg Wt cyclin D1 were treated with 5 μM SHetA2 for 8 h in the presence of 10 μM MG132, a proteasomal degradation inhibitor. Protein extracts (200 μg) were immunoprecipitated with 8 μg of cyclin D1 antibodies and the Western blot was probed with ubiquitin antibody. C, cell cycle profile of cells treated for 24 h with 5 μM SHetA2 in the presence of different concentrations of MG132. Columns, mean (n ≥ 3); bars, SE. D, cell cycle profiles of cultures treated with 5 μM SHetA2 in the presence or absence of 10 mmol/L LiCl or SB216763 (10 μmol/L) for 24 h. Columns, mean (n ≥ 3); bars, SE.
Results

**SHetA2 induces G1-phase cell cycle arrest and cyclin D1 loss independent of p53 and apoptosis in ovarian cancer cells.** The effects of SHetA2 on cell cycle profiles were measured by flow cytometric analysis of three ovarian cancer cell lines that differed by p53 status and origin. A2780, established from an ovarian adenocarcinoma of an untreated patient, expresses Wt p53 (30); SK-OV-3, established from the ascitic fluid of an ovarian cancer patient, has a p53 gene deletion and does not express any p53 mRNA or protein (30); whereas Caov-3, established from an ovarian adenocarcinoma, expresses Mt p53 (31). Despite these differences, all three ovarian cancer cell lines exhibited a dose-dependent increase in the percentage of cells in the G1 phase of the cell cycle (Fig. 1A for A2780 and SK-OV-3; data not shown for Caov-3), indicating that p53 is not required for the mechanism of G1 arrest. Maximal G1 arrest was induced by 5 μmol/L SHetA2, and this concentration was used to treat cells in subsequent experiments. G1 arrest increased in a time-dependent manner (Fig. 1A). A2780 cells responded more rapidly to SHetA2, exhibiting statistically significant G1 arrest after 4 hours (P = 0.00257). Both SK-OV-3 and A2780 cells exhibited significant G1-phase arrest at 16 hours (A2780, P = 0.000712; SK-OV-3, P = 0.00490) and at 24 hours (A2780, P = 0.000346; SK-OV-3, P = 0.00759). This G1 arrest was associated with both a time- and dose-dependent decrease of cyclin D1 protein (Fig. 1B) in SHetA2-treated cultures compared with untreated controls.

Flow cytometric analysis of cells stained with Annexin V and PI was performed to determine if apoptosis was occurring at the 5 μmol/L dose and 24-hour time point that induced maximal G1 arrest. Although this treatment induced a statistically significant reduction in total cell number of cells (A2780, P = 0.00157; SK-OV-3, P = 0.0378; Fig. 1C, left), there was no significant increase in the number of cells undergoing apoptosis (Fig. 1C, right).

**The mechanism of cyclin D1 loss involves T286 phosphorylation, ubiquitination, and proteasomal degradation independent of GSK-3β.** Cyclin D1 levels are frequently up-regulated in cancer and this may be partly due to deregulated proteasomal degradation (32). Western blot analysis of enriched nuclear and cytoplasmic extracts showed that SHetA2 induced phosphorylation of cyclin D1 on T286, which is known to target this protein for ubiquitination (10). This occurred in a time-dependent manner in both fractions with a concomitant decrease in total cyclin D1 (Fig. 2A; results for nuclear extracts not shown). Subsequent increases in cyclin D1 ubiquitination were confirmed after 8 hours of SHetA2 treatment in both cell lines (Fig. 2B). An inhibitor of
proteasomal degradation, MG132, was used to test if SHetA2-induced cyclin D1 loss and G1 arrest are due to enhanced proteasomal degradation. Treatment with SHetA2 in the presence of MG132 totally abrogated the G1 arrest observed in the presence of SHetA2 alone (Fig. 2C). MG132 also partially restored the cyclin D1 in SHetA2-treated extracts (Supplementary Fig. S1A) to levels comparable with treatment with MG132 alone, implicating cyclin D1 degradation in the mechanism of G1 arrest. Simultaneous treatment with LiCl and SB216763 to inhibit GSK-3β, a kinase known to phosphorylate cyclin D1 at T286 (32, 33), did not prevent SHetA2-induced cyclin D1 degradation (Supplementary Fig. S1B) or G1 arrest (Fig. 2D), suggesting that GSK-3β does not mediate the SHetA2-induced cyclin D1 phosphorylation and subsequent events. In contrast, Western blot analysis of both nuclear and cytoplasmic extracts showed that SHetA2 induced phosphorylation of GSK-3β at S9, which inhibits its kinase activity (Supplementary Fig. S1C).

**Cyclin D1 overexpression attenuated SHetA2-induced G1 arrest.** To directly test the role of cyclin D1 in the mechanism of SHetA2-induced G1 arrest, the effects of expression of Wt and T286A, a degradation-resistant Mt, of cyclin D1 on SHetA2 G1 arrest were evaluated. Transfection with either Wt or Mt cyclin D1 caused partial reversal of G1 loss after SHetA2 treatment in comparison with mock-transfected A2780 cells (Fig. 3A). Western blots verified the transfection and cyclin D1 expression (Fig. 3B). The lack of...
complete abrogation of G1 arrest is likely due to low transfection efficiencies in these cell lines. A similar trend was seen in SK-OV-3 cells, although G1 attenuation was less than in the A2780 cells (Fig. 3A). When the percentage of A2780 cells in G1 was compared by one-way ANOVA, the P value was <0.00001, and Tukey’s multiple comparison test for each set of pairs gave the following values: mock versus Wt, P < 0.05; mock versus Mt, P < 0.001; and Wt versus Mt, P < 0.01. In SK-OV-3, the ANOVA P value was <0.001, and for pairwise comparisons, the ANOVA P value was as follows: mock versus Wt, P < 0.05; mock versus Mt, P < 0.001; and Wt versus Mt, P > 0.05. In A2780 cells when compared with the mock-transfected cells, there was greater G1 arrest attenuation in the degradation-resistant cyclin D1 Mt–transfected cells when compared with Wt cyclin D1–transfected cells after treatment with SHetA2. This further supports the role of proteasomal degradation in the SHetA2–induced mechanism.

**SHetA2 alters cyclin E2 levels and p21 interactions.** The effects of SHetA2 on the expression and interaction of additional G1 phase cell regulatory molecules that have been implicated in ovarian cancer, cyclin E2 and p21 (1), were evaluated. Both cyclin E2 and p21 proteins were elevated at the 24-hour SHetA2 treatment time point at protein level (Fig. 4A). Cyclin E2 mRNA levels were also elevated after treatment with SHetA2 in all three ovarian cancer cell lines (Supplementary Fig. S2). Both cyclin D1 and cyclin E2 coimmunoprecipitated with p21 in the untreated control cells (Fig. 4B). In SHetA2-treated cells, cyclin D1 did not coimmunoprecipitate with p21, which was expected because cyclin D1 protein is absent at this treatment time point as shown earlier (Fig. 1B). In addition, greater amounts of cyclin E2 coimmunoprecipitated with p21 in treated cells compared with the untreated control.

**p21 siRNA restores pRb S612 phosphorylation but not G1-S progression.** Both cyclin D1–stimulated and cyclin E2–stimulated cdk activity converges on hyperphosphorylation of pRb; hence, the effect of SHetA2 on the phosphorylation status of pRb was investigated by Western blot (Fig. 4C). SHetA2 caused a reduction in pRb phosphorylation at S780 and S612 but not S795. Because p21 complexed with both cyclin D1 and cyclin E2 in our studies, p21 was predicted to be involved in the mechanism of SHetA2–mediated G1 arrest. Specific siRNA knockdown of p21 did not affect SHetA2–induced G1 arrest in either cell line (Fig. 4D). Knockdown of p21 in SHetA2–treated cells did not prevent SHetA2-mediated loss of phosphorylation of pRb S780 but did partially restore pRb S612 phosphorylation, a site phosphorylated by cyclin E2-cdk2 (Fig. 4D, bottom; ref. 34).

**SHetA2 inhibits E2F transactivation and cyclin A expression.** Decreased phosphorylation of pRb has been shown to inhibit uncoupling of E2F-DP from pRb and to repress E2F transactivation (20). To test this, the effects of SHetA2 on luciferase activity were measured in ovarian cancer cell lines cotransfected with a luciferase reporter construct driven by E2F DNA binding sites. SHetA2 inhibited E2F promoter activity ~ 3-fold (Fig. 5A). To verify that the observed decrease in E2F promoter activity altered expression of E2F target genes, the effects of SHetA2 on endogenous cyclin A levels were determined using Western blot. As expected, cyclin A levels were decreased in SHetA2–treated cells in comparison with control (Fig. 5B).

**Discussion**

The results of this study show that cyclin D1 plays a dominant role in regulating cell cycle progression in ovarian cancer cells and that degradation of cyclin D1 through treatment with SHetA2 is sufficient to induce G1 cell cycle arrest. This conclusion is supported by the demonstration that G1 arrest can be inhibited by overriding the cyclin D1 loss through overexpression of Wt or a nondegradable Mt of cyclin D1 or by inhibiting proteasomal degradation. Whereas cyclin E has been shown to compensate for knockout of cyclin D during fetal development and in other studies (15–17), our studies show that cyclin E2 expression is insufficient to facilitate G1 to S progression in the absence of cyclin D1. Under normal conditions, expression of cyclin E is controlled by cyclin D through activation of cdk4/6 hypophosphorylation of pRb (6), causing the partial release of E2F-AP at the cyclin E promoter from repression by pRb and HDAC (6, 9, 20). In SHetA2–treated ovarian cancer cell lines, however, cyclin E2 continued to be expressed at the 24-hour time point despite complete loss of cyclin D1 at an earlier (8 hours) treatment time point. This suggests that in the ovarian cancer cells studied, cyclin E2 expression may be independent of cyclin D1. Although both cyclin E2 mRNA and protein levels were increased in SHetA2–treated cultures, it cannot be concluded that the effect is a direct effect of SHetA2 on transcription because the increased cyclin E2 levels could be due to the higher percentage of G1-phase cells in the SHetA2–treated cultures that may have contained cyclin E2 protein before treatment.

The inability of cyclin E2 to compensate for cyclin D1 loss can be explained by the molecular events that occur downstream of cyclin
D1 loss in ovarian cancer cells. The SHetA2-induced decrease in phosphorylation of pRb at S780 is consistent with cyclin D1 loss and the report that this site is exclusively phosphorylated by cyclin D-cdk4/6 (34). Decreased phosphorylation of pRb at the S612 site reported to be exclusively phosphorylated by cyclin E-cdk2, however, is not consistent with the continued expression of cyclin E2 observed after SHetA2 treatment (34) but can be explained by p21 repression of cdk2 kinase activity. There has been controversy over the role of p21 in cdk4/6 inhibition; however, the general consensus is that p21 is a positive regulator of cdk4/6 but inhibits cdk2 when complexed with either cyclin E or cyclin A (20, 35, 36).

In this study, coimmunoprecipitation experiments showed that p21 binds both cyclin D1 and cyclin E2. As cyclin D1 is lost in SHetA2-treated cells, and cyclin E2 levels are increased, the increased cyclin E2 is bound by p21 protein (Fig. 6). Repression of cdk2 activity by p21 has been previously reported (35, 36) and is consistent with the ability of p21 siRNA to partially reverse the reduction in pRb S612 phosphorylation caused by SHetA2-induced transfer of p21 from cyclin D1 to cyclin E2. Thus, in untreated ovarian cancer cells, cyclin D1 facilitates cdk2 activity by titrating p21 from cyclin E2-cdk2. In SHetA2-treated cells, increased levels of cyclin E2 are bound and inhibited by increased levels of total p21 and may be supplemented with p21 released from degraded cyclin D1 complexes.

The inability of p21 siRNA to attenuate SHetA2-induced G1 arrest further exemplifies the dominant role of cyclin D1 activity over cyclin E2 activity in ovarian cancer cell lines. As anticipated, p21 siRNA released repression of cyclin E2-cdk2 activity and allowed pRb S612 phosphorylation, but due to the absence of cyclin D1 in SHetA2-treated cells, pRb S780, a cyclin D-cdk4/6 phosphorylation site, remained unphosphorylated in the presence of p21 siRNA. Thus, pRb S612 phosphorylation by cyclin E2-cdk2 is insufficient to overcome loss of cyclin D1 and the resulting reduction of pRb S780 phosphorylation. This dominant role of cyclin D1-cdk4 over cyclin E2-cdk2 in driving cell cycle progression indicates that cyclin D1 is a relevant target for the development of drugs for ovarian cancer.

The SHetA2-induced reduction in pRb S612 and S795 phosphorylation was expected to inhibit release of E2F from pRb. The consequence of decreased release of E2F was confirmed by the observed reduction in E2F transactivation activity and transcription of an E2F endogenous gene, cyclin A. Cyclin A regulates S-phase progression and mitosis because it binds to and activates both cdk2 and cdk1 (37). Thus, the downstream mechanism of SHetA2-induced G1 arrest through cyclin D1 degradation involves both decreased expression of cyclin A and release of p21, which serves to repress cdk2 activity (Fig. 6).

GSK-3β was the first kinase shown to phosphorylate cyclin D1, thus targeting it for nuclear export by CRM1, ubiquitination, and proteasomal degradation (33). In the ovarian cell lines used in this study, however, cyclin D1 T286 phosphorylation and degradation is independent of GSK-3β because two separate GSK-3β inhibitors failed to abrogate cyclin D1 degradation. Instead, SHetA2 induced phosphorylation of GSK-3β at S9, an event known to inhibit the kinase activity (33, 38). These observations suggest the existence of another redundant kinase for which cyclin D1 (T286) acts as a substrate.

The ability to inhibit the deregulated cell cycle progression of ovarian cancer cell lines through a specific molecular mechanism supports the application of SHetA2 in ovarian cancer chemoprevention. SHetA2 offers promise in this regard in that it has not exhibited toxicities in multiple animal models, including models of ovarian cancer xenograft growth, skin irritancy, and teratogenicity (22, 26, 27). Further evidence for SHetA2 chemoprevention activity includes the reversal of carcinogen-induced transformation in an in vitro organotypic model and in vivo inhibition of angiogenesis and xenograft growth (23, 27). The demonstration in this study that SHetA2 induces G1 arrest in all cell lines tested regardless of the status of p53 suggests that it will be effective against a broad spectrum of tumor types, including tumors that have lost p53 function. The tumor suppressor gene p53 is the most commonly mutated gene in all cancers. The efficacy of SHetA2 is not limited to ovarian cancer because it can inhibit growth of a wide variety of cancers (22).

Figure 6. Model. In normal cells, mitogenic stimuli induce cyclin D expression. Cyclin D binds to cdk4/6, forming a complex, and titrates p21 from the cell, facilitating pRb hypophosphorylation. This results in partial derepression of the E2F transcription factor, allowing expression of cyclin E. Cyclin E forms a complex with and activates cdk2, which further hyperphosphorylates pRb, resulting in total derepression of the E2F promoter. This allows transcription of S-phase genes that are under the control of the E2F promoter. In cancer cells, there is aberrant cyclin D1 and cyclin E2 expression. When SHetA2 is added to the cells, cyclin D1 is lost through proteasomal degradation. This loss results in recruitment of p21 to cyclin E2-cdk2 complexes, inhibiting cdk2 activity. This prevents pRb hyperphosphorylation and the E2F promoters remain repressed by the bound pRb complex, resulting in G1 arrest and inhibition of cyclin A expression.
In this study, it is shown that cyclin D1 is a dominant regulatory protein of cell cycle progression and a potential chemopreventive and chemotherapeutic agent of ovarian cancer. Hence, the low-toxicity SHetA2 compound shows promise as a chemopreventive agent for ovarian cancer and other types of cancers. This is significant because several molecules that inhibit cyclin D1 or the cognate kinase cdk4/6 are in clinical development and agents that down-regulated cyclin D1 resulted in positive outcomes (39–41). In addition, cyclin D1 seems to be clinically predictive of cancer risk (39), suggesting that cyclin D1 levels can be used as an end point biomarker for both chemoprevention and chemotherapy. Whereas cyclin D1 proteasomal degradation is the primary biochemical end point of SHetA2-induced G1 arrest, increased cyclin E2 and p21 levels, alterations in cyclin D1-ckd4/6-p21 and cyclin E2-ckd2-p21 protein complexes, decreases in pllb S780 and S612 phosphorylation, and decreased expression of cyclin A are secondary biochemical end points. Some of these downstream effects of SHetA2 may be useful surrogate biomarkers of treatment responsiveness and efficacy before clinical evidence of treatment response.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

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