Advances in Brief

Resveratrol Induces Prostate Cancer Cell Entry into S Phase and Inhibits DNA Synthesis

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Abstract

Resveratrol has an apoptotic effect on a variety of cancer cells. Changes in cell cycle regulatory processes contributing to the antiproliferative effect of resveratrol remain largely unknown. Our studies revealed that, in androgen-sensitive LNCaP cells, the effect of resveratrol on DNA synthesis varied dramatically depending on the concentration and the duration of treatment. In 1-h-treated cells, resveratrol showed only an inhibitory effect on DNA synthesis, which increased with increasing concentration ($IC_{50} = 20 \mu M$). However, when treatment duration was extended to 24 h, we observed a dual effect of resveratrol on DNA synthesis. At 5 to 10 μM it caused a 2- to 3-fold increase in DNA synthesis, and at $\geq 15 \mu M$, it inhibited DNA synthesis. The increase in DNA synthesis was seen only in LNCaP cells, but not in androgen-independent DU145 prostate cancer cells or in NIH3T3 fibroblast cells. The resveratrol-induced increase in DNA synthesis was associated with enrichment of LNCaP cells in S phase, and a concurrent decrease in nuclear $p21^{Cip1}$ and $p27^{Kip1}$ levels. Furthermore, consistent with the entry of LNCaP cells into S phase, there was a dramatic increase in nuclear Cdk2 activity associated with both cyclin A and cyclin E. Taken together, our observations indicate that LNCaP cells, treated with resveratrol, are induced to enter into S phase, but subsequent progression through S phase is limited by the inhibitory effect of resveratrol on DNA synthesis, particularly at concentrations above 15 μM. Therefore, this unique ability of resveratrol to exert opposing effects on two important processes in cell cycle progression, induction of S phase and inhibition of DNA synthesis, may be responsible for its apoptotic and antiproliferative effects.

Introduction

With the increase in life expectancy and modern methods of diagnosis, prostate cancer has become the most frequently diagnosed cancer in males in the United States (1). The incidence of prostate cancer in the United States is 30-fold greater than in Osaka, Japan, and 120-fold greater than in Shanghai, China (2). The low incidence of prostate cancer in Asian men is attributed by some to dietary consumption of bioflavonoids, including phytoestrogens (3, 4). Therefore, a full understanding of phytoestrogen action on cell cycle regulatory processes may lead to their effective use in chemoprevention and/or treatment of prostate cancer.

Resveratrol is a phytoestrogen present in the skin of red grapes and various other food products, with structural similarity to estradiol and diethylstilbestrol (5). Each gram of fresh red grape skin contains 50–100 μg of resveratrol and is, therefore, abundant in red wine with concentrations ranging from 10 to 20 μM (6, 7). Oral administration of 28 μg of resveratrol to male rats achieves a peak plasma level of resveratrol greater than 20 ng/ml after 1 h (8). This phytoestrogen has anticarcinogenic activity by preventing initiation, promotion, and progression of skin cancer in mice (7). The phytoestrogen activity of resveratrol has been inferred to account for its effects on hormone-regulated events contributing to cell proliferation and viability. In breast cancer cells, resveratrol has an antiproliferative effect by antagonizing the stimulatory effects of estrogen (9, 10). In human prostate cancer cells, resveratrol has an antiproliferative effect by disrupting the G_1-S phase transition and inducing apoptosis (11). At 25 μM, resveratrol lowered the expression and secretion of prostate-specific antigen, by an AR^3-independent mechanism, without affecting the expression of AR (11, 12). However, at higher concentrations (>50 μM), resveratrol inhibited both the expression and function of AR (13). Prostate-specific antigen, human glandular kallikrein-2 (hK2), the steroid receptor coactivator ARA70, and Cdk inhibitor p21, were also repressed by resveratrol at either the mRNA or protein level in LNCaP cells (13). Despite these insights, changes in cell cycle regulatory events contributing to antiproliferative effects of resveratrol remain largely unknown.

In an effort to understand molecular events contributing to the antiproliferative effect of resveratrol, we investigated its effects on hormone-sensitive, AR-positive LNCaP and hormone-independent, AR-negative DU145 prostate cancer cells at concentrations that are pharmacologically relevant and at treatment intervals when its effects are most noticeable. Our data show that resveratrol has a biphasic effect in LNCaP cells, depending on the concentration and duration of treatment. When LNCaP cells are treated for 24 h, it has both a stimulatory and inhibitory effect on DNA synthesis depending on the concentration. The stimulatory effect on DNA synthesis is associated with enrichment of LNCaP cells in S phase. On the other hand, in DU145 cells, it shows only an inhibitory effect on DNA synthesis. These unique features of resveratrol provide important clues to the molecular basis for its antiproliferative effect on prostate cancer cells, and suggest its potential usefulness as an adjuvant in chemotherapy of prostate cancer.

Materials and Methods

Cell Culture. LNCaP, DU145, and NIH3T3 cells were obtained from the American Type Culture Collection, Manassas, VA. LNCaP cells were grown in RPMI 1640 (Life Technologies, Inc., Rockville, MD) supplemented with 10% FCS, 100 μg/ml of streptomycin, 100 units/ml penicillin, 1 μg/ml hydrocortisone, and 100 nM testosterone; and DU145 and NIH 3T3 cells were grown in DMEM (Life Technologies, Inc.) containing 10% FCS, streptomycin. and penicillin, in a humidified incubator with 5% CO_2 and 95% air at 37°C.

Resveratrol Treatment. Exponentially growing cells (3–4 days after plating at 2×10^4/well) in a 12-well plate were treated with resveratrol. Resveratrol stock (100 mM in DMSO) was diluted 100-fold into PBS containing 20% ethanol. An appropriate volume of working stock (1 mM) was then added to the

[3] The abbreviations used are: AR, androgen receptor; FACS, fluorescence-activated cell sorting; Cdk, cyclin-dependent kinase; ER, estrogen receptor.

cells to achieve the indicated final concentrations of resveratrol. Control cells received the same volume of diluent.

Measurement of [³H]Thymidine and [5-³H]Uridine Incorporation. After 1- or 24-h treatment with resveratrol, cells were pulse-labeled with [³H]thymidine or [5-³H]uridine (2 μCi/ml) for 30 min at 37°C in a humidified incubator. The radioactivity incorporated into acid-precipitable material was determined as described by Reddy (14). Under these conditions, there is exclusive incorporation of [³H]thymidine into DNA and of [5-³H]uridine into RNA.

Cell Cycle Analysis. Exponentially growing cells were plated in 150-mm Petri dishes and treated with l0 μM or 20 μM resveratrol. After 24 h, the cells were harvested, fixed with 70% ethanol at a cell density of 0.7×10^6 cells/ml, and stored overnight at 4°C. Cell cycle distribution was analyzed by flow cytometry using the FACS analysis core services of Henry Ford Health Sciences Center, Detroit, MI. The FACS analysis procedure involved washing the fixed cells once in HBSS (Life Technologies, Inc.) and then treating them with HBSS-containing propidium iodide (50 μg/ml) and RNase A (5 units/ml). Within 30 min after the treatment, cell samples were subjected to flow cytometry using a BD LSR instrument (Becton Dickinson, San Jose, CA) equipped with a 488-nm (blue) argon and a 32-nm (UV) helium-cadmium laser. Data acquisition was performed using CellQuest software and data analysis with ModFit LT (2.0) software (Variety Software House, Inc., Topsham, ME).

Western Blot Analysis. Nuclear fractions of cells treated with 10 or 25 μM resveratrol were prepared as described by Subramanyam *et al.* (15). An equal amount of protein in each fraction was subjected to denaturing PAGE (SDS-PAGE), and the proteins resolved on the gels were transferred to nitrocellulose membrane. Individual membranes were then probed with antibodies specific to p21[Cip1], p27[Kip1], Cdk2, Cyclin A, Cyclin B, or Cyclin E (Santa Cruz) and immunoreactive bands on the membrane were detected by using alkaline phosphatase conjugated secondary antibodies as described elsewhere (16). Relative absorbance of the immunoreactive bands in each gel was determined by first capturing their images using the Eagle Eye II Still Video System (Stratagene, La Jolla, CA) and then analyzing the images using Multi-Analyst software (Bio-Rad, Hercules, CA). Background absorbance at regions adjacent to the immunoreactive bands on the filters was subtracted from the absorbance of the bands. Density of the immunoreactive bands in resveratrol treated samples was then expressed as the percentage of the density of corresponding bands in control samples. Density of bands in control samples is treated as 100%.

Immunoprecipitation and Kinase Assay. Fifty μg of protein from each nuclear fraction, prepared as described above, was diluted in 0.3 ml of immunoprecipitation buffer [50 mM Tris-HCl (pH 7.4), 0.1% Triton X-100, 5 mM EDTA, 250 mM NaCl, 50 mM NaF, and 0.1 mM Na$_3$VO$_4$] supplemented with protease inhibitor mixture (catalogue no. P-8340, Sigma Chemical Co) and incubated overnight with 3 μg of either anti-cyclin A or anti-cyclin E rabbit polyclonal antibodies (Santa Cruz Biotechnology). Protein-A/G beads (Pierce, Rockford, IL; 20 μl) were then added to the incubation mixture and the incubation continued for one more h. Immunoprecipitate-bound protein-A/G beads were washed three times with immunoprecipitation buffer, and the nuclear proteins remaining bound to the protein-A/G beads were then analyzed by histone H1 kinase assay for the presence of Cdk2 activity and by Western blot method for the presence of cyclins A or cyclin E, Cdk2, p21[Cip1], and p27[Kip1].

The histone H1 kinase activity of Cdk2 in immunoprecipitates was measured by first washing the protein-A/G beads three times with kinase buffer [50 mM HEPES, (pH 7.5), 10 mM MgCl$_2$, and 1 mM DTT] and incubating them with 20 μM ATP, 10 μCi [γ-³²P]-ATP, and 5 μg of histone H1 in a final volume of 30 μL at 37°C for 20 min. The reaction was terminated by adding 7.5 μL of 5× sample buffer [300 mM Tris-HCl (pH 6.8), 10% SDS, 50% glycerol, 3 mg/ml bromphenol blue]. The reaction mixture was then boiled and subjected to SDS-PAGE, transferred to nitrocellulose membrane, and exposed to X-ray film at −70°C to detect the radioactive band of ³²P-labeled histone H1.

Results

Biphasic Effect of Resveratrol on DNA Synthesis in LNCaP Cells. To determine proliferation-related effects, we selected a concentration range (2–30 μM) of resveratrol that showed no evidence of

apoptosis after 24-h treatment of LNCaP cells (data not shown). In cells treated with resveratrol for 1 h, there was an inhibitory effect on DNA synthesis that increased in a dose-dependent fashion (Fig. 1). The concentration of resveratrol required for 50% inhibition of DNA synthesis, compared with controls, was ~20 μM. However, 24-h treatment of LNCaP cells with 2–30 μM resveratrol had a biphasic effect on [³H]thymidine incorporation into DNA. A 24-h treatment with resveratrol at low concentrations (5–10 μM) stimulated (2- to 3-fold), and at high concentrations (15–30 μM) inhibited (2- to 4-fold) DNA synthesis, compared with controls (Fig. 1). Thus, depending on the concentration and duration of treatment, resveratrol showed both inhibitory as well as stimulatory effects on DNA synthesis in LNCaP cells.

Preferential Effect of Resveratrol on DNA Synthesis. To evaluate the specificity of the resveratrol effect on DNA synthesis, we also examined its effects on RNA synthesis in LNCaP cells. DNA synthesis, measured by [³H]thymidine incorporation, was stimulated by low concentrations of resveratrol (5–10 μM) and inhibited by high concentrations of resveratrol (15–30 μM). By contrast, RNA synthesis, measured by [5-³H]uridine incorporation, was not affected by either low or high concentrations of resveratrol (Fig. 2). Therefore, resveratrol shows a preferential effect on DNA synthesis in LNCaP cells, even when compared with its effect on a closely related macromolecule (RNA) synthesis.

Stimulatory Effect of Resveratrol on DNA Synthesis Is Unique to Androgen-sensitive LNCaP Cells. To test whether the stimulatory effect of 5–10 μM resveratrol on DNA synthesis is a phenomenon common to all cell types, we compared the effect of resveratrol on DNA synthesis in androgen-sensitive AR-positive LNCaP and androgen-independent AR-negative DU145 prostate epithelial cells, and fibroblastic NIH3T3 cells. We observed that 5–10 μM resveratrol stimulated DNA synthesis only in LNCaP cells, but not in DU145 or NIH 3T3 cells. In both DU145 and NIH3T3 cells, which lack AR, resveratrol only inhibited DNA synthesis in a dose-dependent manner (Fig. 3).

Resveratrol Recruits LNCaP Cells into S Phase. In search of a potential basis for the observed increase in thymidine incorporation in LNCaP cells treated with 10 μM resveratrol for 24 h, we performed flow cytometry analysis. As shown in Fig. 4, we observed more than a 2-fold increase in the number of cells in S phase after treatment with 10 μM resveratrol for 24 h as compared with the controls. Even at a
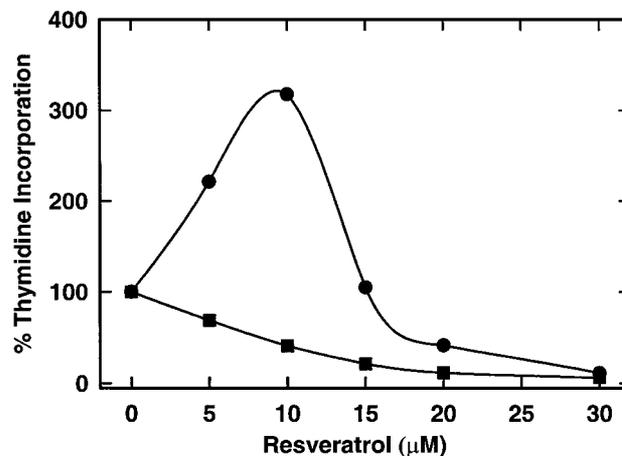

Fig. 1. Effect of 1-h and 24-h resveratrol treatment on [³H]thymidine incorporation into DNA of LNCaP cells. Exponentially growing cells were treated with various concentrations of resveratrol for either 1 h (■) or 24 h (●) as described in "Materials and Methods." They were then pulse-labeled with [³H]thymidine for 30 min, and radioactivity incorporated into DNA was measured.
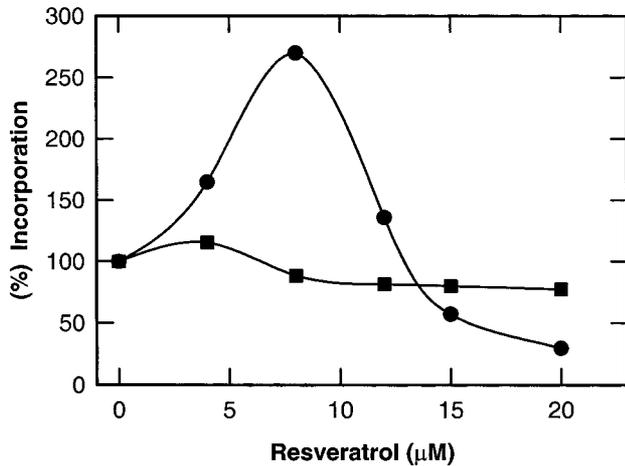
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Fig. 2. Effect of resveratrol on [^{3}H]thymidine and [5-^{3}H]uridine incorporation into LNCaP cells. Cells treated with resveratrol were pulse-labeled with [^{3}H]thymidine (●) or [5-^{3}H]uridine (■) and were processed for measuring radioactivity incorporated into nucleic acids as described in "Materials and Methods."
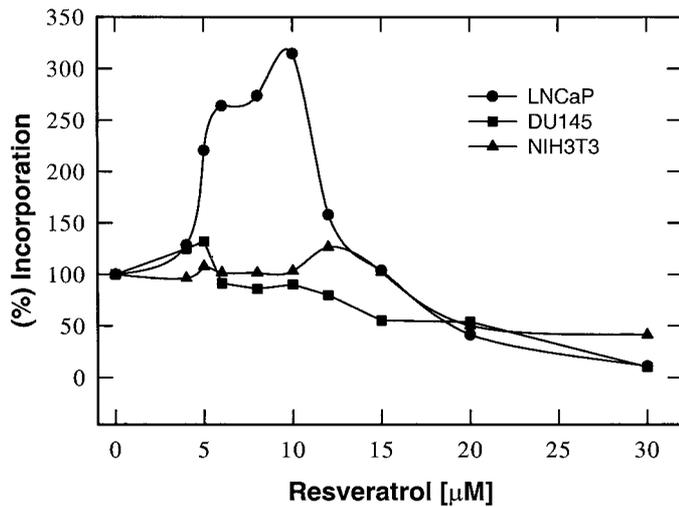

Fig. 3. Comparison of resveratrol effect on [^{3}H]thymidine incorporation in androgen-sensitive (LNCaP), androgen-independent (DU145) prostate epithelial cells, and NIH 3T3 fibroblast cells. LNCaP (●) and DU145 (■) prostate epithelial cells, and NIH 3T3 fibroblast cells (▲) were plated similarly and treated with resveratrol for 24 h, and [^{3}H]thymidine incorporation into DNA was measured as described in "Materials and Methods."

higher concentration (20 μM), when resveratrol has an inhibitory effect on DNA synthesis, there was a greater proportion of cells in S phase compared with untreated cells (Fig. 4). Resveratrol also caused a significant decrease in the G_2-M population of cells after treatment for 24 h.

Resveratrol Represses Nuclear p21^{Cip1} and p27^{Kip1} Levels in LNCaP Cells. To assess the role of cell cycle regulatory proteins in resveratrol-induced entry of LNCaP cells into S phase, we examined the nuclear distribution of several pertinent cell cycle regulatory proteins in LNCaP cells after treatment with resveratrol for 24 h. As shown in Fig. 5, resveratrol caused a concentration-dependent decrease in nuclear p21^{Cip1} and p27^{Kip1}, inhibitors of Cdks. None of the other cell cycle regulatory proteins, including Cdk2, cyclin A, cyclin B, and cyclin E, tested in the same nuclear extracts showed any consistent changes in their levels, which suggests that the observed decrease in nuclear p21^{Cip1} and p27^{Kip1} levels is related to the ability of prostate cancer cells to enter into S phase and is unlikely to be an artifact of cell fractionation procedures used for the preparation of nuclear extracts.

Resveratrol Increases Nuclear Cyclin A-Cdk2 and Cyclin E-Cdk2 Activities. Twenty-four-h treatment of LNCaP cells with resveratrol also resulted in a dramatic increase in nuclear Cdk2 activity associated with both cyclin A and cyclin E (Fig. 6A). This increase in Cdk2 activity in nuclear extracts of resveratrol-treated cells is not an artifact of the immunoprecipitation procedure because there were no significant differences in the level of either cyclin A or cyclin E in the corresponding immunoprecipitates (Fig. 6B). As in nuclear lysates, p21^{Cip1} and p27^{Kip1} were significantly lower in the immunoprecipitates derived from resveratrol-treated cells than in those from controls (Fig. 6B). The resveratrol-induced changes in Cdk2 activity associated with both cyclin A and cyclin E are likely the net result of interaction among Cdk2, p21^{Cip1}, and p27^{Kip1} in the complex. Furthermore, it is interesting to note that, whereas the cyclin E-Cdk2 activity increased with increasing concentration of resveratrol, the increase in cyclin A-Cdk2 activity was much higher in 10 μM resveratrol-treated cells than in cells treated with 25 μM resveratrol (Fig. 6A).

Discussion

The present study demonstrates for the first time the dose- and treatment duration-dependent biphasic effect of resveratrol on DNA synthesis in androgen-sensitive LNCaP prostate cancer cells. After

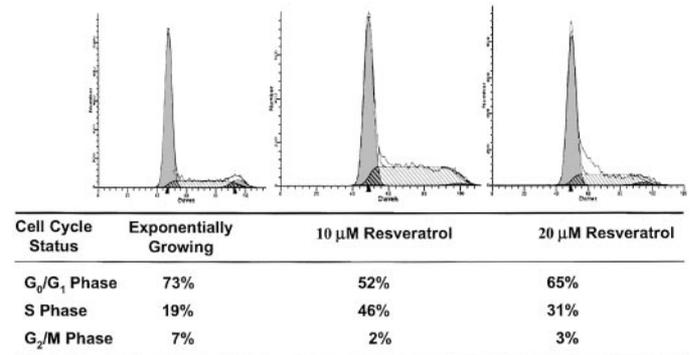| Cell Cycle Status	Exponentially Growing	10 μM Resveratrol	20 μM Resveratrol
G_0/G_1 Phase	73%	52%	65%
S Phase	19%	46%	31%
G_2/M Phase	7%	2%	3%

Fig. 4. Cell cycle analysis of exponentially growing LNCaP cells treated with resveratrol. Exponentially growing LNCaP cells were treated with diluent (control) or with 10 μM or 20 μM resveratrol as described in "Materials and Methods." After 24 h, the cells were prepared for flow cytometry analysis as described in "Materials and Methods." The table shows the percentage of G_1, S phase, and G_2-M populations of cells under each treatment condition. These data are representative of at least three independently repeated experiments.
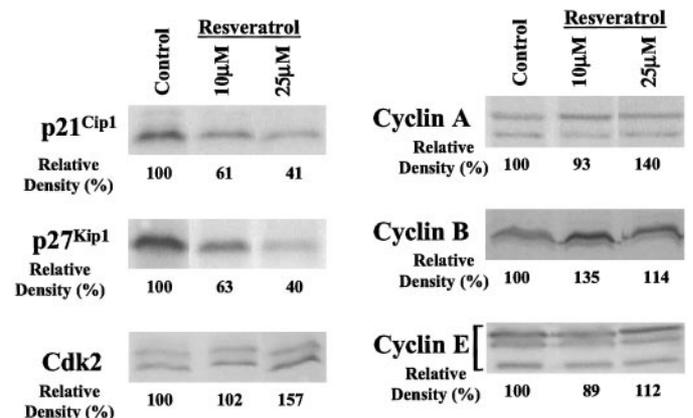

Fig. 5. Effect of resveratrol on the nuclear cell cycle regulatory proteins in LNCaP cells. Exponentially growing LNCaP cells were treated with the indicated concentrations of resveratrol for 24 h. Nuclear lysates were prepared and immunoblot analysis using monoclonal antibodies to detect p21^{Cip1}, p27^{Kip1}, Cdk2, and cyclins A, B, and E was performed as described in "Materials and Methods." Profiles of cell cycle regulatory proteins are representative of at least three independently repeated experiments.

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A) Histone H1 kinase activity
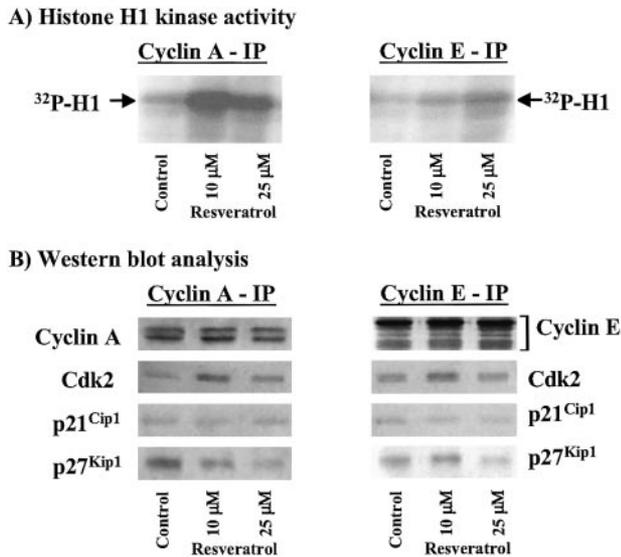

B) Western blot analysis

Fig. 6. Effect of resveratrol on Cdk2 activity associated with cyclin A and cyclin E in LNCaP cells. Exponentially growing LNCaP cells were treated with 10 or 25 μM resveratrol. Cyclin A- and cyclin E-specific immunoprecipitates were prepared from nuclear lysates, and Cdk2 activity was measured by histone H1 kinase assay (*A*), and p21^{Cip1}, p27^{Kip1}, cyclin A, and cyclin E were measured by Western blot analysis (*B*) in each of the immunoprecipitates as described in "Materials and Methods." Immunoprecipitates recovered from equal amount of protein (25 μg) in individual nuclear lysates were used in these assays. Results are representative of at least two independently repeated experiments.

24-h treatment, resveratrol at low concentrations (5–10 μM) induced the entry of cells into S phase, resulting in a 2- to 3-fold higher rate of DNA synthesis as compared with controls, and at high concentrations (>15 μM) it inhibited DNA synthesis. This biphasic effect of resveratrol was seen only in androgen-sensitive LNCaP cells, but not in androgen-independent DU145 prostate cancer cells nor in NIH3T3 fibroblast cells. The biphasic effect of resveratrol that was evident in LNCaP cells after 24-h treatment was not seen when the treatment duration was reduced to 1 h (Fig. 1). A 1-h treatment with resveratrol showed only an inhibitory effect on DNA synthesis that increased in a dose-dependent fashion. Irrespective of the duration of treatment, in our study, the concentration of resveratrol that was required for 50% inhibition of DNA synthesis, as compared with the controls, was 20 μM. This is consistent with the study by Jang *et al.* (7), who reported a resveratrol IC$_{50}$ of 18 μM for inhibition of [^{3}H]thymidine incorporation in HL-60 cells.

Hsieh and Wu (11) reported that resveratrol induces apoptosis without disrupting the ability of LNCaP cells to transit through G$_1$-S phase. A similar observation was made with HL-60 (17) and breast cancer cells (18). However, under the conditions used in the present study, resveratrol did not show any apoptotic effect on LNCaP cells (data not shown) and the inhibitory effect of 25 μM resveratrol on DNA synthesis, which was observed even after 24-h treatment, was completely reversible (data not shown). A similar reversible inhibitory effect of resveratrol was reported in studies with HL-60 cells (19).

Our observation that the 2- to 3-fold increase in DNA synthesis occurs only when cells were treated with resveratrol for 24 h but not for 1 h (Fig. 1) suggests that the entry of cells into S phase involves signaling pathways that act over several hours. Although the signaling pathways affected by resveratrol are not known, it is intriguing to note that resveratrol also induces the proliferation of ER-positive KLP-1 and MCF-7 breast cancer cells (18), which suggests a role of ER in signaling. Resveratrol at 10–25 μM has been reported to function as an estrogen agonist to increase the growth of ER-positive breast cancer cells (20). Resveratrol exhibits a variable degree of ER ago-

nism depending on the cell type (5). ER expression in prostate cancer is controversial (21–23). However, a point mutation in the ligand-binding domain of the AR in LNCaP cells makes AR responsive to estrogenic stimuli (24). It remains to be seen whether resveratrol-induced entry of LNCaP cells into S phase involves steroid hormone receptor signaling.

Flow cytometry analysis of LNCaP cells in our study showed a dramatic enrichment of LNCaP cells in S phase when treated with 10 μM resveratrol for 24 h (Fig. 4). We observed that, after treatment with resveratrol, there was a significant decrease in the nuclear p21^{Cip1} and p27^{Kip1} levels. Mitchell *et al.* (13) observed a similar effect of resveratrol on p21^{Cip1} in LNCaP cells. Considering that it is the nuclear component of cell cycle regulatory proteins that plays a critical role in determining the ability of cells to progress through the cell cycle and enter into S phase (25, 26), the decrease in nuclear p21^{Cip1} and p27^{Kip1} in resveratrol-treated LNCaP cells in the present studies may account for their increased ability to enter into S phase. In the present studies with 10–25 μM resveratrol, when cells were actively recruited into S phase, there was actually a significant decrease in the G2/M population as compared with control (Fig. 4). A similar observation was made in studies with HL-60 cells (19). As described below, this limitation of cells to enter into G$_2$-M is likely a consequence of the inhibitory effect of resveratrol on DNA synthesis in S phase.

Cyclin A-Cdk2 activity, which normally is expressed during mid-to-late S phase (27), increased dramatically after resveratrol treatment (Fig. 6). However, its increase was relatively higher in LNCaP cells treated with 10 μM resveratrol than in those treated with 25 μM resveratrol (Fig. 6). This perhaps reflects an efficient progression of cells through S phase in the presence of low concentrations (5–10 μM) of resveratrol as compared with those in the presence of high concentration (>15 μM) of resveratrol (Fig. 4). In the presence of high concentrations (20–25 μM) of resveratrol, cells were also induced to enter into S phase (Fig. 4), possibly because of an increase in nuclear cyclin E-Cdk2 activity (Fig. 6), which is associated with the progression of cells from G$_1$ into S phase (27). However, their subsequent progression through S phase seems to be limited by a direct inhibitory effect of resveratrol on some of the key enzymes of DNA synthesis, such as DNA polymerase α and δ (28, 29) and ribonucleotide reductase (30). Such a limitation of cells to progress through S phase may result in fewer mid-to-late S-phase cells, and, therefore, a decreased accumulation of cyclin A-Cdk2 in the presence of 25 μM resveratrol than in the presence of 10 μM (Fig. 6). This direct inhibitory effect of resveratrol on the enzymes of DNA synthesis could also be responsible for the observed inhibition of DNA synthesis in 1-h-treated cells (Fig. 1), and the decrease in G$_2$-M phase population after 24-h treatment (Fig. 4). Thus, resveratrol seems to create a collision course between two important processes in cell cycle progression by activating signaling pathways required for the entry of cells into S phase and at the same time limiting their progression through S phase by inhibiting DNA synthesis. This collision course between cell cycle regulatory processes during a prolonged treatment with resveratrol may be responsible for resveratrol-induced cell death observed in some of the earlier studies in which cells were treated with resveratrol for several days (11, 20, 31).

The unique ability of resveratrol to recruit prostate cancer cells into S phase observed in the present studies also raises the possibility of its usefulness in chemotherapy. Because prostate cancer is a slow-growing disease, only a small fraction of its cells are actively proliferating at any given time. This poses a problem for its treatment, because a majority of currently available chemotherapeutic agents and ionizing radiation are most effective in killing the cells that are actively proliferating. Therefore, any agent that induces proliferative stimula-

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tion of prostate cancer cells can increase their sensitivity to the cytotoxic effects of chemotherapeutic agents and ionizing radiation. In this regard, a preferential effect of resveratrol in recruiting androgen-sensitive prostate cancer cells into S phase makes it a potentially attractive adjuvant for chemotherapy or ionizing radiation therapy to target proliferating prostate cancer cells in S phase. Furthermore, based on its ability to effectively inhibit DNA synthesis in both androgen-sensitive and androgen-independent prostate cancer cells, resveratrol may also prove to be useful in delaying the progression of prostate cancer. Thus, these studies for the first time demonstrate a biphasic, dual effect of resveratrol on cell cycle regulatory processes that play an important role in determining cell viability.

Acknowledgments

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