

Differential efficacy of 3-hydroxy-3-methylglutaryl CoA reductase inhibitors on the cell cycle of prostate cancer cells

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Abstract

Members of the statin family of 3-hydroxy-3-methylglutaryl CoA reductase inhibitors are being investigated for the therapy and prevention of cancers because of their growth-inhibitory effects on epithelial cells. Some epidemiologic studies show that patients taking statins show a lower incidence of cancer compared with those taking other cholesterol-lowering medication. In contrast, other studies show that statin use does not correlate with cancer risk. To address this discrepancy, we investigated the efficacy of different statins on the PC-3 prostate cancer cell line and the androgen-dependent LNCaP prostate cancer cell line. Clinically used statins, lovastatin, fluvastatin, and simvastatin inhibit proliferation of the two prostate cancer cells by inducing a G₁ arrest. Lovastatin induced the arrest at 0.5 μmol/L, a concentration easily reached in the serum after oral administration. Pravastatin, however, was less effective at inhibiting 3-hydroxy-3-methylglutaryl CoA reductase in PC-3 cells and had to be present at 200 times higher concentrations to effect a cell cycle arrest. Another potential source of variability is the different levels of the cyclin-dependent kinase (cdk) inhibitor p27 noted in prostate cancers particularly because statins have been suggested to act through the induction of cdk inhibitors. All three statins (lovastatin, fluvastatin, and simvastatin) inhibited cyclin E/cdk2 kinase leading to hypophosphorylation of Rb, but this inhibition was correlated with a loss of the activating phosphorylation on Thr¹⁶⁰ of cyclin E-associated cdk2 and not dependent on the cdk inhibitors p21 and p27. Therefore, p27 status is unlikely to confound the

epidemiologic data on the efficacy of statins in prostate cancer. To make definitive conclusions about the efficacy of statins on cancer prevention, however, the epidemiologic studies should take into account the type of statin used and the serum concentrations achieved and ensure that the tested statin inhibits the specific type of cancer *in vitro* at those concentrations. [Mol Cancer Ther 2006;5(9):2310–6]

Introduction

In 2006 alone, >230,000 new cases of prostate cancer will be diagnosed and >27,000 lives will be lost to this disease. There is a need for rapid and effective preventive or curative therapies for this cancer. Statins are a family of drugs that inhibit 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase), the enzyme catalyzing the rate-limiting step in cholesterol biosynthesis. Statins bind to and sterically block the hydroxymethyl glutarate-binding site on the enzyme (1). These drugs have been used effectively over the last decade in the treatment of hypercholesterolemia.

Recently, statins are being studied as potential cancer therapeutics. As early as 1992, xenografts of pancreatic cancer cells in nude mice showed growth inhibition of the cancer cells after lovastatin treatment (2). More recent epidemiologic studies support further investigation into the effectiveness of statins as anticancer therapeutics (reviewed in ref. 3). These studies have shown a decreased risk of developing cancer in general (4, 5) or in specific cases of prostate, breast, or hepatic cancers (AACR meeting, 2005; refs. 6, 7). These observations make the statins attractive candidates for cancer therapy or prevention. Several recent studies, however, contradict this and find that there is no direct correlation between statin use and cancer incidence (8–10). We therefore wanted to examine whether all clinically used statins were equally effective at inhibiting the proliferation of prostate cancer cells. We addressed this by testing the ability of a panel of statins to arrest PC-3 prostate cancer cells. We have previously shown that PC-3 cells are arrested in G₁ after mevastatin treatment (11). The use of clinically relevant statins provides a biochemical framework by which to judge epidemiologic studies on cancer prevention by statins. Three of the four statins inhibited G₁-S transition at moderate doses. Pravastatin, the weakest inhibitor of HMG-CoA reductase, was required at 200-fold excess concentration for cell cycle effects, suggesting that the preventive effects of statins on cancer emergence may vary with specific statins. Statins have been suggested to block G₁-S transition via the induction of the cyclin-dependent kinase (cdk) inhibitors p21 and p27 (12–14). Because

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a significant fraction of prostate cancers have activated mechanisms to degrade p27 (15, 16), this could be another cause of variable epidemiologic findings. We therefore examined the importance of p27 in the cell cycle arrest. The statins that effectively arrested PC-3 cells all had the same cell cycle effects, including inhibition of cyclin E/cdk2 kinase and dephosphorylation of cdk2 on Thr¹⁶⁰. This effect, however, was independent of p21 and p27 accumulation. Together, these results suggest that, to make definitive conclusions about the effects of statins on prostate cancer, the epidemiologic studies should take into account the dose and the type of statin used for treatment.

Materials and Methods

Reagents and Tissue Culture

Antibodies to p21 (C-19), p27 (C-19), cdk2 (M2), cyclin E (HE12 for immunoblotting and HE111 for immunoprecipitation), cyclin A (H432), cdk4 (C-22), p130 (C-20), and hemagglutinin (Y-11) were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-Rb, phosphorylated Rb, and cdk2 Thr¹⁶⁰ antibodies were purchased from Cell Signaling Technology (Beverly, MA). Anti-E2F1 was purchased from Upstate Biotechnology (Waltham, MA), and anti- β -actin antibody was purchased from Sigma (St. Louis, MO). Lovastatin, pravastatin, fluvastatin, and simvastatin were purchased from Calbiochem (San Diego, CA). Mevastatin, lovastatin, fluvastatin, and simvastatin were dissolved in DMSO (Sigma). Pravastatin was prepared in water. PC-3 and LNCaP cells were obtained from the American Type Culture Collection (Manassas, VA) through the University of Virginia Tissue Culture Facility. Cells were grown in RPMI 1640 with L-glutamine (Invitrogen, Grand Island, NY) supplemented with 10% fetal bovine serum (Irvine Scientific, Santa Ana, CA) and 1% penicillin/streptomycin (Invitrogen). Cells were treated with the indicated concentration of the various statins for 48 hours. [γ -³²P]ATP was purchased from Perkin-Elmer Life Sciences (Boston, MA).

Immunoblotting

Cells treated with statins were harvested after 48 hours and lysed using buffer containing 50 mmol/L Tris-HCl (pH 7.4), 0.1% NP40, 150 mmol/L NaCl, 5 mmol/L EDTA, 50 mmol/L NaF, 1 mmol/L Na₃VO₄, and protease inhibitors. Total protein (30 μ g) was loaded for each sample and resolved by SDS-PAGE. Proteins were transferred to nitrocellulose membranes, and immunoblotting was done as per the protocols provided by the respective companies from whom antibodies were purchased.

Flow Cytometry

Cells were fixed with 70% ethanol, washed with PBS, and then stained with propidium iodide. Fluorescence-activated cell sorting (FACS) analysis was done on a Becton Dickinson (San Jose, CA) FACSCalibur benchtop cytometer at the University of Virginia Flow Cytometry Core Facility.

Plasmid Transfection

PC-3 cells were transfected with a hemagglutinin-tagged Ha-RasV12-expressing vector using LipofectAMINE 2000 (Invitrogen) following their standard protocol. The cells

were then treated with the indicated doses of the appropriate statin or DMSO. After 48 hours, the cells were harvested and lysed as described. The cell lysates were then resolved on a 12% SDS-PAGE gel and probed with anti-hemagglutinin antibody.

Immunoprecipitation and Kinase Assays

Cyclin E was immunoprecipitated from 1 mg of total protein lysates overnight and then pulled down on protein G-Sepharose beads for 2 hours. The beads were washed five times with lysis buffer and then resuspended in SDS sample buffer. For cyclin E-associated kinase assays, 100 μ g protein was immunoprecipitated as described above. The beads were washed thrice with lysis buffer and then twice with kinase buffer [50 mmol/L HEPES-NaOH (pH 7.4), 25 mmol/L MgCl₂]. The beads were then incubated with 25 μ L kinase reaction mixture [50 mmol/L HEPES-NaOH (pH 7.4), 25 mmol/L MgCl₂, 0.5 mmol/L DTT, 50 μ mol/L ATP, 5 μ Ci [γ -³²P]ATP, 2 μ g histone H1]. The reactions were incubated at 30°C for 30 minutes. The reaction was stopped by the addition of 12.5 μ L of 3 \times SDS sample buffer.

RNA Interference Transfections

The p21 small interfering RNA (siRNA) oligonucleotide targets the 3'-untranslated region of p21 at position 1,941 (AACAUACUGGCCUGGACUGUU; refs. 11, 17). The p27 siRNA oligonucleotide targets the 3'-untranslated region at position 2,304 (AAGGUUGCAUACUGAGCCAAG; ref. 18). A control GL2 siRNA oligonucleotide (19) was used. After the second siRNA transfection, the cells were treated with either DMSO or lovastatin for 48 hours. The cells were then harvested and used for flow cytometry, or lysates were prepared for immunoblotting as described above.

Results

Pravastatin Does Not Behave Like Lovastatin, Fluvastatin, or Simvastatin in PC-3 Cells

We have previously shown that mevastatin can arrest PC-3 cells in the G₁ phase of the cell cycle (11). We wanted to test whether different statins currently in clinical use also caused cell cycle arrest in PC-3 prostate cancer cells. In addition to mevastatin (11), PC-3 cells were treated with 10 μ mol/L of lovastatin, pravastatin, fluvastatin, or simvastatin for 48 hours. Lovastatin, fluvastatin, and simvastatin all arrested PC-3 cells in G₁ to the same extent as mevastatin. However, at this dose, pravastatin did not have any effect on the cell cycle (Fig. 1).

To ensure that the pravastatin was active, we tested the ability of the drug to inhibit HMG-CoA reductase by assessing the inhibition of Ras cleavage. Ras proteins are post-translationally processed by isoprenylation and COOH-terminal cleavage to form the mature active Ras (20). Inhibition of isoprenoid biosynthesis using fluoromevalonate inhibited the cleavage of Ras and produced a slower-migrating form of Ras (21). Inhibition of HMG-CoA reductase by statins inhibits the synthesis of isoprenyl moieties required for Ras activation and results in the

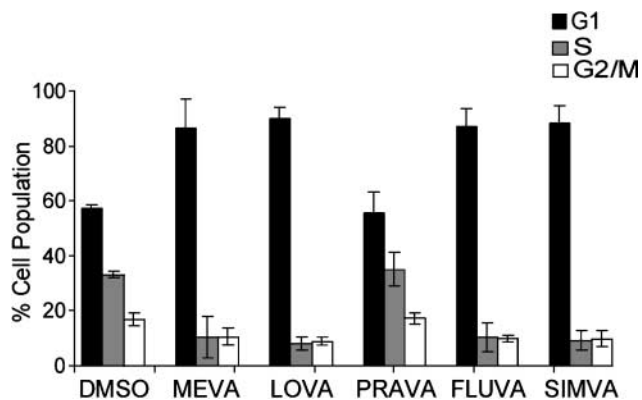


Figure 1. Statins have differential efficacy against PC-3 cells. FACS analysis of cells 48 h after treatment with statins. Cells were treated with 10 $\mu\text{mol/L}$ of mevastatin (MEVA), lovastatin (LOVA), pravastatin (PRAVA), fluvastatin (FLUVA), or simvastatin (SIMVA), fixed in ethanol, stained with propidium iodide, and analyzed by flow cytometry. DMSO treatment was used as a control. Percentage of cells in G₁ (black columns), S (gray columns), and G₂-M (white columns). Columns, mean of three experiments; bars, SD.

appearance of the precursor form of Ras. We transfected PC-3 cells with a hemagglutinin-tagged constitutively active form of H-RasV12 that still requires isoprenylation and cleavage for activation. The cells were then treated with various doses of lovastatin and pravastatin. In control cells, only a single form of mature Ras can be detected. However, after treatment with lovastatin or pravastatin, a higher molecular weight precursor form of Ras was detected (Fig. 2, *top*), indicating that Ras was no longer being post-translationally processed. Lovastatin caused inhibition of Ras cleavage and cell cycle arrest (Fig. 2, *bottom*) even at 0.5 $\mu\text{mol/L}$ concentration. No inhibition of Ras cleavage or inhibition of cell proliferation was detected with pravastatin at <20 $\mu\text{mol/L}$ concentration (data not shown). At 20 $\mu\text{mol/L}$ concentration, inhibition of Ras cleavage was not efficient and there was no inhibition of cell proliferation. At least 200 $\mu\text{mol/L}$ pravastatin was required before the cells started to arrest in G₁ (Fig. 2, *bottom*). Thus, pravastatin is the least effective of the statins to inhibit proliferation of PC-3 prostate cancer cells.

All the Statins, except Pravastatin, Target Cell Cycle Regulators Similarly in PC-3 Cells

We next examined the effect of statins on the steady-state levels of various cell cycle regulatory proteins involved in the G₁-S transition (Fig. 3). In all cases where statin treatment caused G₁ arrest, Rb was hypophosphorylated and so migrated faster on the SDS-PAGE gel. Immunoblotting with antibodies that specifically detect Rb phosphorylation at positions 807 and 811 showed that phosphorylation at these two sites was decreased with all statins, except pravastatin. E2F1 was decreased after statin treatment. The decrease in E2F1 protein was accompanied by a decrease in the mRNA (data not shown). Because the E2F1 promoter is autoregulated by E2F, the hypophosphorylated Rb is expected to repress E2F and down-regulate the E2F1 mRNA. The hypophosphorylation of Rb

and the decrease in E2F1 predict that the downstream targets of E2F1 (cyclins E and A) will be down-regulated. Although there was no change in cyclin E levels, cyclin A protein levels were decreased. Activation of cyclin promoters has been suggested to occur in a stepwise fashion with cyclin D/cdk4 directed phosphorylation of Rb being sufficient to derepress the cyclin E promoter (22), whereas cyclin E/cdk2 activity is necessary to derepress the cyclin A promoter (23, 24). Thus, the decrease of cyclin A, but not cyclin E, suggests that the specific kinase activity of cyclin E/cdk2 is inhibited in the statin-treated cells. cdk2 is the kinase that partners with cyclins E and A and migrates as a doublet with the phosphorylated active cdk2, migrating faster than the dephosphorylated inactive form (25). The total cellular pool of cdk2 was dephosphorylated after statin treatment (Fig. 3). However, the cdk2-activating kinase only phosphorylates cyclin-associated cdk2 so that the dephosphorylation of the total pool of cdk2 can be explained by the absence of cyclin A and the attendant increase in free cdk2. These results suggested that the primary defect in the cell cycle was at the level of Rb phosphorylation and E2F activation somewhere between cyclin E synthesis and cyclin A synthesis. In addition, as with the FACS analysis, pravastatin had very little effect on the cell cycle regulators at the concentrations tested.

Lovastatin, Fluvastatin, and Simvastatin Cause Cyclin E-Associated cdk2 Dephosphorylation and Inhibition of cdk2 Activity

We have previously shown that mevastatin treatment specifically causes cyclin E-associated cdk2 dephosphorylation on Thr¹⁶⁰ (11). We therefore measured the specific

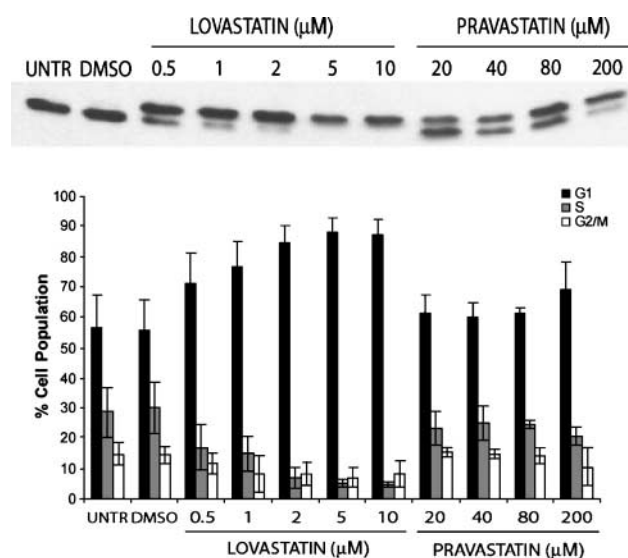


Figure 2. *Top*, pravastatin is functional as an inhibitor of HMG-CoA reductase because it can inhibit the cleavage of precursor Ha-RasV12 to the mature form. PC-3 cells transfected with Ha-RasV12 were then treated with DMSO or with various concentrations of lovastatin or pravastatin. *Bottom*, at higher concentrations, pravastatin causes G₁ arrest. FACS analysis of PC-3 cells treated with DMSO, lovastatin, or pravastatin. Columns, mean of three experiments; bars, SD.

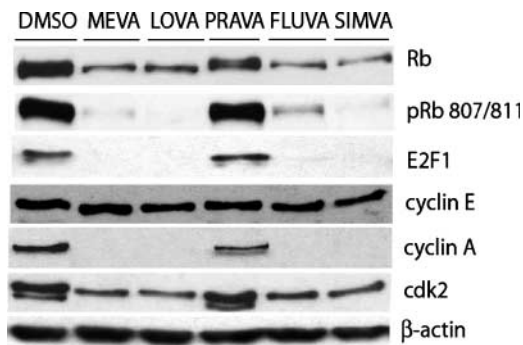


Figure 3. Immunoblots of various cell cycle regulators 48 h after treatment of PC-3 cells with 10 $\mu\text{mol/L}$ concentration of various statins. Right, protein detected. The Rb antibody detects both unphosphorylated and hyperphosphorylated (slower migrating) Rb. The pRb807/811 detects Rb phosphorylated at Ser⁸⁰⁷/Ser⁸¹¹.

activity of cyclin E-associated cdk2 kinase activity *in vitro* after treatment with the other statins. The specific activity of cyclin E-associated kinase was decreased in cells arrested in G₁ by statins (Fig. 4, bottom). cdk2 activity depends on the addition of the activating phosphorylation at Thr¹⁶⁰ and the removal of the cdk inhibitors p21 and p27. We first examined the phosphorylation of the cyclin E-associated cdk2 fraction by immunoprecipitating cyclin E and detecting cdk2 by immunoblotting. The amount of cyclin E immunoprecipitated was similar in all the samples (Fig. 4, top). In normal cells, the cyclin E-associated cdk2 is predominantly phosphorylated on Thr¹⁶⁰ and, therefore, faster migrating (25). After statin treatment, a significant portion of the cyclin E-associated cdk2 was dephosphorylated and, thus, inactive as indicated by the appearance of a slower-migrating cdk2 band in the statin-treated samples (except with pravastatin; Fig. 4, middle). The defect therefore seems to be at the level of cyclin E/cdk2 activation.

The Cell Cycle Effects of Statins Are Independent of p21 and p27

Because studies have proposed that the statin-induced G₁ arrest is dependent on accumulation of the cdk inhibitors p21 and p27 (12–14) and because these inhibitors could repress cyclin E/cdk2 kinase activation, we examined the levels of these two proteins. p21 levels were significantly increased after statin treatment (Fig. 5A; ref. 11). There was also an increase in p27 levels with the clinically used statins, leading us to wonder whether p21 and p27 were redundant with each other at inhibiting cyclin E/cdk2. We therefore tested whether codepletion of both p21 and p27 would abrogate the cdk2 kinase inhibition. p21 and p27 were depleted using siRNA targeting the mRNA of these genes. Both protein levels were reduced to <10% of the control cells after siRNA transfection (Fig. 5B). The cell cycle effects of lovastatin were examined under conditions with low p21 and p27. Depletion of p21 and p27 in the absence of statins caused an increase in the basal levels of phosphorylated Rb and

cyclin A protein (Fig. 5B, lane 1 versus lane 3). However, after statin treatment, Rb was still hypophosphorylated and cyclin A protein levels were still reduced. Cyclin E-associated cdk2 kinase activity continued to be inhibited after statin treatment despite the reduction of p21 and p27 levels in the cells (Fig. 5C). The cells continued to arrest in G₁ after statin treatment even in the absence of p21 and p27 (Fig. 5D). Therefore, the accumulation of cdk inhibitors is not critical for the inactivation of cdk2 and the other observed cell cycle effects, and the p27 status of a prostate cancer is unlikely to impinge on the inhibitory effects of the statins.

Statins Also Induce G₁ Arrest and Cyclin E-Associated cdk2 Dephosphorylation in LNCaP Cells

We next examined the effect of statins on the cell cycle profile and regulatory proteins in the androgen-dependent LNCaP prostate cancer cells. LNCaP cells also arrested in G₁ after lovastatin treatment but not after pravastatin treatment (Fig. 6A). The mechanism of G₁ arrest in LNCaP cells seems similar to that in PC-3 cells (Fig. 6B and C). Rb is hypophosphorylated, and phosphorylation at positions 807 and 811 is decreased (Fig. 6B). The pRb-associated protein E2F1 decreases with a concomitant decrease in its mRNA level (data not shown). There is very little change in cyclin E levels, whereas cyclin A levels decrease. The decrease in E2F1 and cyclin A is consistent with these two promoters being regulated by E2F1.

The total cellular cdk2 pool was dephosphorylated as indicated by a decrease in the faster-migrating cdk2 band (Fig. 6B). Based on our results with PC-3 cells, we examined the phosphorylation status of cyclin E-associated cdk2. After statin treatment, about half the cyclin E-associated cdk2 was dephosphorylated on Thr¹⁶⁰ (Fig. 6C, middle). Consistent with the dephosphorylation of cyclin E-associated cdk2, the cyclin E-associated kinase activity was decreased as measured by an *in vitro* kinase assay (Fig. 6C, bottom). Thus, the same statins that cause G₁ arrest in PC-3 cells also arrest LNCaP cell proliferation by inhibiting cyclin E/cdk2 activity at a step after the assembly of the kinase but before activation through phosphorylation on Thr¹⁶⁰.

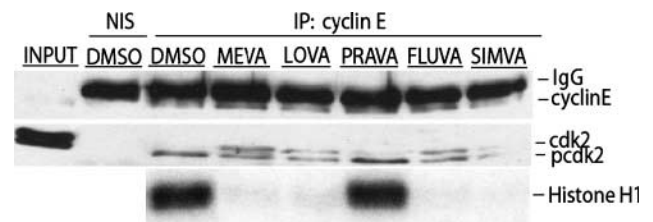


Figure 4. Cyclin E-associated cdk2 immunoblot and kinase activity in PC-3 cells after treatment with 10 $\mu\text{mol/L}$ concentration of various statins. Total cellular protein (1,000 μg) was used to immunoprecipitate cyclin E. Immunoprecipitated samples were resolved on a 12% SDS-PAGE gel and then probed for cyclin E or cdk2. Protein (1%) from untreated cells was used as input. Immunoprecipitated cyclin E was also used to carry out a histone H1 kinase assay (as described in Materials and Methods).

None of these cell cycle effects were seen after treatment with pravastatin, the weakest inhibitor of HMG-CoA reductase and inhibitor of cell proliferation (data not shown).

Discussion

The goal of this study was to investigate whether different statins show varying efficacy in inhibiting prostate cancer cell proliferation. Mevastatin (a lovastatin homologue) was used as a positive control because it has previously

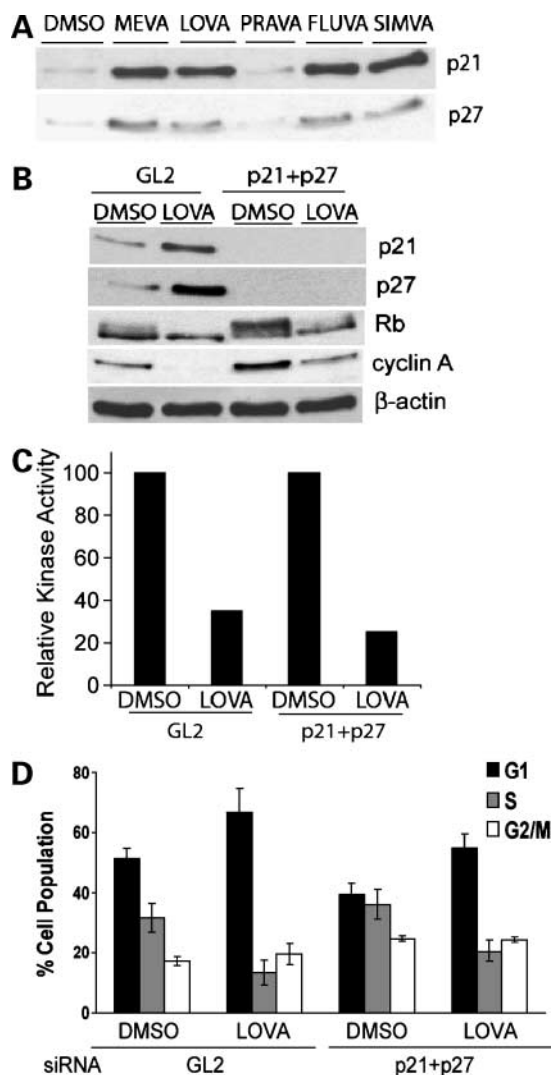


Figure 5. **A**, Western blotting of p21 and p27 48 h after treatment of PC-3 cells with 10 $\mu\text{mol/L}$ dose of various statins. **B**, Western blotting for p21, p27, Rb, cyclin A, and β -actin after siRNA against p21 and p27. A siRNA against GL2 was used as a control. **C**, cyclin E-associated kinase activity after p21 and p27 siRNA in PC-3 cells. The activity in statin-treated cells is represented as a percentage of the DMSO-treated sample in each case. Results are an average of two independent experiments. **D**, FACS analysis of PC-3 cells treated with DMSO or lovastatin following siRNA-mediated depletion of p21 and p27. Results are an average of two independent experiments.

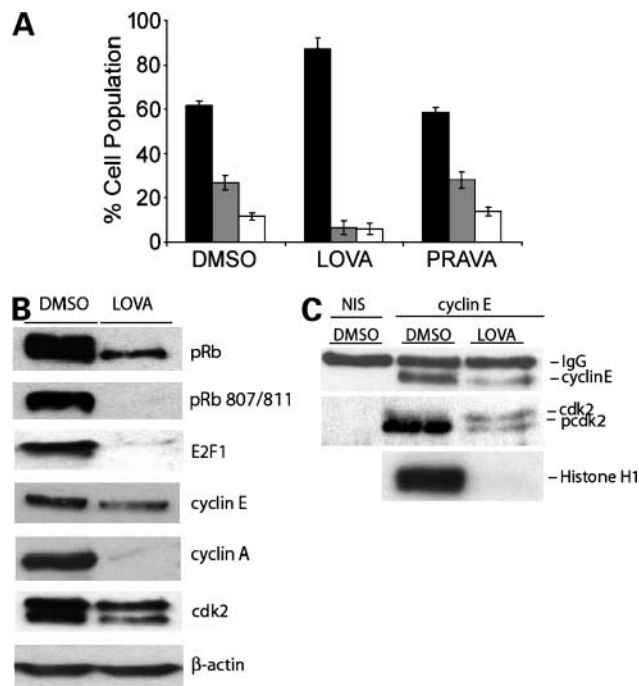


Figure 6. **A**, statins also have differential efficacy against LNCaP cells. FACS analysis of cells 48 h after treatment with statins. Cells were treated with lovastatin or pravastatin, fixed in ethanol, stained with propidium iodide, and analyzed by flow cytometry. DMSO treatment was used as a control. Percentage of cells in G₁ (black columns), S (gray columns), and G₂-M (white columns). Columns, mean of three experiments; bars, SD. **B**, immunoblots of various cell cycle regulators 48 h after treatment of LNCaP cells with DMSO or lovastatin. **Right**, protein detected. **C**, statins also inhibit cyclin E-associated cdk2 activity in LNCaP cells. Cyclin E-associated cdk2 immunoblot and kinase activity in LNCaP cells after treatment with DMSO or lovastatin. See Materials and Methods for details.

been used by us to inhibit cell proliferation (11, 17). Lovastatin, fluvastatin, and simvastatin arrest all the prostate cancer cells in the G₁ phase of the cell cycle. The cell cycle effects of pravastatin required at least 200 times higher concentration than lovastatin, consistent with the weaker effect of this statin on cholesterol decrease in prostate cells (Fig. 2, bottom; ref. 26). Pravastatin also inhibits HMG-CoA reductase less efficiently in PC-3 cells based on the ability of the drug to inhibit Ras activation (Fig. 2, top). Together, these results suggest that there is a correlation between HMG-CoA reductase inhibition and cell cycle effects. Similar effects were also observed in the androgen-dependent prostate cancer cell line LNCaP (Fig. 6), suggesting that statins may be effective in both androgen-dependent and androgen-independent cancer therapies.

It is worth noting that pravastatin may not be as effective as the other statins in preventing or treating cancers because the inhibition of cell proliferation is seen at doses where there could be potentially toxic side effects. In contrast to pravastatin, lovastatin arrests cells at G₁ even at 500 nmol/L concentrations. In animal models, doses of 2 $\mu\text{mol/L}$ lovastatin are well tolerated over a period of months (27). Clinical trials on lovastatin indicate that

doses of 25 mg/kg/d did not cause serious side effects and, at doses of 4 mg/kg/d, serum concentrations could reach $\sim 4 \mu\text{mol/L}$ (28). Because we see cell cycle arrest at $0.5 \mu\text{mol/L}$ lovastatin (Fig. 2), therapeutic doses of lovastatin to inhibit prostate cancer can be easily achieved. In contrast, the levels of pravastatin normally observed in serum are lower than the doses required for cell cycle effects (29, 30).

The differential efficacy of statins could also explain the conflicting epidemiologic results on cancer incidence (3–6, 8–10, 31). Several contributing factors need to be considered. The first issue is the tissue being considered in the study. Pravastatin was found to be very effective in prolonging the life of patients with hepatocellular carcinomas (6). This correlates very well with the efficacy with which pravastatin inhibits cholesterol biosynthesis in the liver ($\sim 90\%$) of mice (26). However, the cholesterol inhibition in the prostate was $<14\%$. As we show in Fig. 2, the efficacy of the statin in causing growth arrest is correlated to its ability to inhibit HMG-CoA reductase activity. Therefore, pravastatin may not be as effective in inhibiting prostate cancer cell proliferation but might be very good at inhibiting hepatic cancer cell proliferation.

A second factor to be considered is the statin being used. The results with pravastatin are clear—it does not inhibit prostate cancer cell proliferation *in vitro*. Because pravastatin is hydrophilic (a methyl side chain is substituted with a more hydrophilic hydroxyl group in pravastatin), it is possible that its uptake into cells is not as efficient as the other statins. Alternatively, pravastatin could be more efficiently metabolized within the cells, inactivating it before any cell cycle effects are observed. Under the *in vitro* conditions used in this study, lovastatin, fluvastatin, and simvastatin are all efficacious in inhibiting prostate cancer cell proliferation. Translating these results to an *in vivo* system might face similar obstacles as pravastatin. One tissue might metabolize a particular statin more efficiently than the others, resulting in a less effective therapeutic dose. Therefore, it might be appropriate for studies to take into account the statin being used and the tissue-specific cancer being studied before correlating statin use to cancer incidence.

In conclusion, it is clear that clinically useful statins, such as lovastatin, fluvastatin, and simvastatin, can inhibit prostate cancer cell proliferation *in vitro* and should be examined for a beneficial effect in epidemiologic studies of prostate cancer incidence and progression. However, it is important that each of the statins be considered separately to avoid confounding effects from statins that are not as effective at inhibiting cell proliferation, such as pravastatin.

Statin are being studied for both the prevention and the therapy of cancers. However, there does not seem to be a common mechanism by which statins inhibit cancer cell proliferation. Previous work has indicated that, in some breast cancer cells, G_1 arrest is induced via inhibition of the proteasome by the closed-ring prodrug form of lovastatin, resulting in the accumulation of the cdk inhibitors p21 and p27 (13). Thus, inhibition of the proteasome could be

another common mechanism by which these diverse effects are mediated. However, all our studies were conducted using the open-ring sodium salt form of lovastatin. We still observed cell cycle arrest with lovastatin compared with pravastatin. Additionally, we have shown that depleting both p21 and p27 does not affect the statin-mediated inhibition of cyclin E-associated cdk2 activity, Rb dephosphorylation, or the cell cycle arrest (Fig. 5). Thus, inhibition of the proteasome by statins could be cell type specific and is unlikely to explain the G_1 arrest in prostate cancer cells. Likewise the p27 status of the prostate cancer is likely to be unimportant for inhibition by lovastatin. The study also raises important biochemical questions: all the statins that affect the cell cycle at low doses have the same effect on cell cycle regulators (Figs. 3 and 4). Cyclin E-associated cdk2 is dephosphorylated and inactivated, and this happens independent of p21 and p27. This effect is also observed in the androgen-dependent LNCaP cell line. What is the mechanism by which statins inhibit cdk2 phosphorylation? Could this mechanism be extended to other prostate cancer cell lines as well? Work is currently under way to address these biochemical questions.

Acknowledgments

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