Orlistat Is a Novel Inhibitor of Fatty Acid Synthase with Antitumor Activity

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ABSTRACT

One of the fundamental principles of pharmacology is that most drugs have side effects. Although considerable attention is paid to detrimental side effects, drugs can also have beneficial side effects. Given the time and expense of drug development, it would be particularly exciting if a systematic method could be applied to reveal all of the activities, including the unappreciated actions, of a potential drug. The present study takes the first step along this path. An activity-based proteomics strategy was used to simultaneously identify targets and screen for their inhibitors in prostate cancer. Orlistat, a Food and Drug Administration-approved drug used for treating obesity, was included in this screen. Surprisingly, we find a new molecular target and a potential new application for Orlistat. Orlistat is a novel inhibitor of the thioesterase domain of fatty acid synthase, an enzyme strongly linked to tumor progression. By virtue of its ability to inhibit fatty acid synthase, Orlistat halts tumor cell proliferation, induces tumor cell apoptosis, and inhibits the growth of PC-3 tumors in nude mice.

INTRODUCTION

Most drugs have side effects. These range in magnitude from simple nuisances to life-threatening complications. Although focus is placed on negative side effects, beneficial side effects are also observed. Unfortunately, the unanticipated effects of a drug are often revealed in the later stages of development or even after the drug has been approved for use. Given the time and expense of drug development, it would be particularly exciting if all activities of a compound could be revealed at the outset of its development. With such information, care could be taken to minimize detrimental side effects, and testing of the drug could be expanded to other indications should its activity profile warrant.

The ability to perform all of the encompassing screens of the activity of a drug may be on the horizon. In principle, one could predict the effects of a drug by knowing all of its targets. Recent emphasis on global profiling strategies, including gene expression profiling and proteomics, drives this type of thinking (1). Yet these profiling technologies measure abundance, not function, and they fall short of making it possible to screen drugs against a plethora of targets. Recent work in the area of chemical biology points the way toward direct profiling of protein activity, offering a possible solution to this hurdle. Two groups have created chemical probes that react at the active site of multiple enzymes of a given class. Liu *et al.* (2) synthesized a probe containing fluorophosphonate as the warhead and biotin as the reporter, and then used this probe to reveal the serine hydrolase activity profile in biological samples. Greenbaum *et al.* (3) showed that the cysteine proteinases profile could be visualized with probes containing reactive epoxides. Because activity-based probes bind at an active site of the enzyme, a direct measure of the level of active enzyme can be obtained. Consequently, it becomes possible to use straightforward competition assays to screen for inhibitors of all of the enzymes within a family.

Here we apply the activity-based screening strategy to identify serine hydrolases in prostate cancer cells. The activity-based nature of the screen also allows us to identify inhibitors of these enzymes. Of particular interest is fatty acid synthase (FAS), which is up-regulated in the prostate cancer (PCa) cells compared with normal prostate epithelial cells, and has been implicated in the progression of various types of tumors (4–9). Interestingly, Orlistat is a novel and rather selective inhibitor of FAS in tumor cells. This drug inhibits the thioesterase function of the enzyme, interferes with cellular fatty acid synthesis, and can halt tumor cell proliferation and induce tumor cell apoptosis. Orlistat also inhibits the growth of PC-3 prostate tumors *in vivo*. Altogether the study reaffirms the significance of FAS in tumor progression and underscores the fact that this enzyme is a valid oncology target. The study also indicates that compounds with reactive β-lactones, such as Orlistat, should be evaluated as potential antitumor agents.

MATERIALS AND METHODS

Activity Profiling of Serine Hydrolases. LNCaP, DU-145, and PC-3 cell lines (American Type Culture Collection) were maintained in RPMI 1640 (Irvine Scientific) supplemented with 10% fetal bovine serum at 37°C in 5% CO_2. The PrEC cell line (Clonetics) was maintained in defined medium supplied by Clonetics. Each cell line was maintained in 150-mm tissue culture dishes. To generate protein lysates, cells were washed with ice-cold PBS and harvested by scraping with a cell lifter into cold PBS. Cells were collected by centrifugation, resuspended in 50 mM Tris-Cl (pH 8.0), and then lysed by sonication as described previously (2, 10). Soluble and insoluble cell fractions were separated by ultracentrifugation for 1 h at 64,000 rpm at 4°C. Protein concentrations were determined by BCA assay (Pierce).

Activity profiling was performed with fluorophosphonate (FP)-polyethylene glycol (PEG)-6-carboxytetramethylrhodamine (TAMRA) using methods described previously (2, 10). Briefly, soluble fractions (40 μl; 1 mg/ml) were treated with 2 μM FP-PEG-TAMRA for 1 h at ambient temperature. Reactions were stopped by the addition of Laemmli buffer and boiling. Nonspecific reaction of the probe was determined with a duplicate sample boiled for 10 min before labeling with FP-PEG-TAMRA. The labeled samples were resolved by 10% SDS-PAGE and visualized by scanning with a Hitachi flatbed scanner at 605 nm.

Serine hydrolase activity in whole cells was measured with a membrane-permeable probe, FP-BODIPY. After addition of Orlistat, the probe was added to cells (final concentration of 2 μM), and the reaction was allowed to proceed to completion (1 h). Cells were lysed by the addition of Laemmli sample buffer and boiled; samples were resolved on SDS-PAGE and visualized by scanning with a Hitachi flatbed scanner at 605 nm.

Inhibition of Serine Hydrolase Activity by β-Lactones. Ebelactone A and B stocks were made in DMSO. Orlistat (Roche) was solubilized from pills in absolute ethanol. Cell lysates were generated at 1 mg/ml as described above. Samples (40 μg) were incubated with inhibitors for 20 min, and FP-PEG-TAMRA was added and reacted for an additional 30 min.

Identification of Labeled Serine Hydrolases. To identify serine hydrolases, a fluorophosphonate probe linked to biotin was used (2, 10). Cell lysates were preadsorbed to avidin-agarose to reduce nonspecific binding of proteins during the purification. Cell lysates were labeled with FP-PEG-biotin (5 μM) for 1 h at room temperature. Protein was separated from unincorporated FP-PEG-biotin by gel filtration on Nap 25 columns. SDS was added to the eluate to a concentration of 0.5%, and the sample was denatured by boiling. Samples were diluted with 50 mM Tris (pH 7.5) and 150 mM NaCl, and

incubated with avidin-agarose for 1 h at room temperature. The agarose beads were washed eight times with 50 mM Tris (pH 7.5) and 150 mM NaCl containing 1% Tween 20. Labeled protein was eluted with Laemmli buffer containing 1% SDS. Protein was resolved by 10% SDS-PAGE and detected by silver staining. Specific bands were extracted and subjected to in-gel digestion by trypsin and peptide mass fingerprinting with matrix-assisted desorption ionization-time of flight as described previously (11, 12).

Expression of the Recombinant Thioesterase Domain of Fatty Acid Synthase. The portion of the FAS gene (gi:21618359) encoding the thioesterase domain was amplified by PCR using the following primers: 5′ ATG ACG CCC AAG GAG GAT GGT CTG GCC CAG CAG (corresponds to nucleotides 6727–6756) and 3′ GCC CTC CCG CAC GCT CAC GCG TGG CT (corresponds to nucleotides 7625–7650). The recombinant thioesterase domain was cloned into pTrcHis (Invitrogen) and expressed in *Escheria coli*. The recombinant protein corresponds to residues 2202 through 2509 of FAS. The thioesterase was purified by Ni-affinity chromatography, and analyzed for activity and inhibition by Orlistat, using methods described above.

Detection of Fatty Acid Synthase by Western Blot. PC-3 cells (5×10^4) treated with Orlistat were boiled in Laemmli buffer, resolved by SDS-PAGE, and transferred to nitrocellulose. The membrane was blocked with nonfat milk and probed with an anti-FAS monoclonal antibody (PharMingen). Binding was visualized with an horseradish peroxidase-conjugated rabbit antimouse IgG (Bio-Rad) followed by chemiluminescent detection with the Western Lighting Chemiluminescence Reagent (Perkin-Elmer).

Inhibition of Fatty Acid Synthesis by Orlistat. Cellular fatty acid synthesis was measured by the incorporation of [^{14}C]acetate (13, 14). Cells (2.5×10^4 cells/well in 24-well plates) were washed twice with PBS and incubated in defined serum-free medium containing 300 μg/ml BSA and insulin, transferrin, and selenium as supplements. Medium was added to the cells in the presence or absence of Orlistat. Cells were incubated with Orlistat for up to 2 h before the addition of 1 μCi of [^{14}C]acetate. Cells were incubated with [^{14}C]acetate for 2 h, at which time medium was removed, and the cells were washed with PBS/EDTA and trypsinized. Cell pellets were washed twice more with PBS, and fatty acids were extracted with chloroform-methanol (1:1) for 30 min. The extract was dried under N$_2$ and extracted with water-saturated butanol. Butanol was evaporated under N$_2$, and labeled fatty acids were detected by scintillation counting.

Effects of Orlistat on Cell Proliferation. PC-3 cells were exposed to Orlistat along with different concentrations of palmitate for 48 h. Fresh medium, along with Orlistat and palmitate, were added every 24 h. Prolifer-
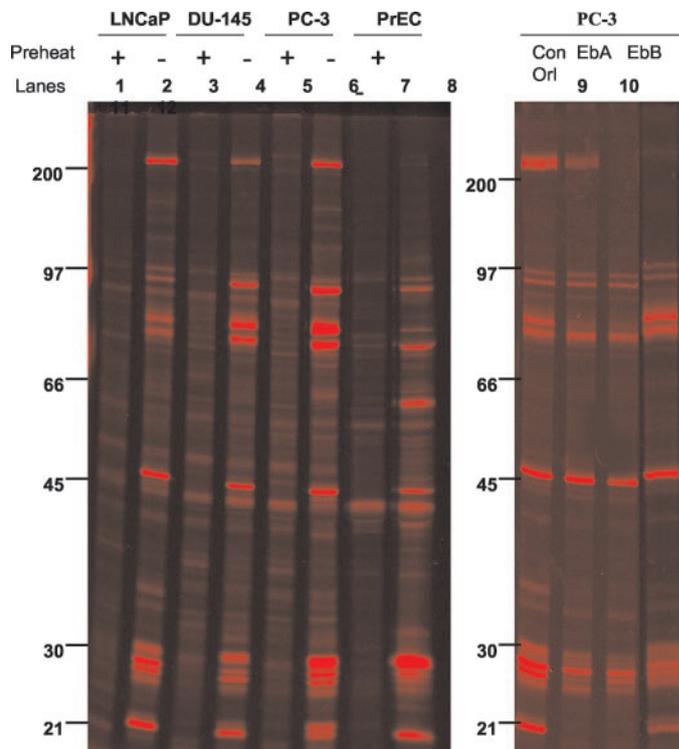

Fig. 2. Activity profiling of normal and neoplastic prostate epithelial cells. Lysates were generated from primary cultures of normal prostatic epithelial cells (*PrECs*) and from three prostate tumor cell lines (*LNCaP, DU-145,* and *PC-3*). Lysates were incubated with fluorophosphonate-polyethylene glycol-6-carboxytetramethylrhodamine (FP-PEG-TAMRA) for 1 h at room temperature. Nonspecific labeling with the activity probe was measured in samples denatured by boiling (lanes marked +). Samples were resolved by 10% SDS-PAGE and visualized at 605 nm using a Hitachi flatbed gel scanner (*lanes 1–8*). The effect of three β-lactones on the activity labeling of serine hydrolases from prostate cancer cells was assessed in a similar manner. Before incubation with FP-PEG-TAMRA, lysates were preincubated with ebelactone A (*lane 10*), ebelactone B (*lane 11*), or Orlistat (*lane 12*). After labeling with FP-PEG-TAMRA, the reactions were halted and enzyme activity visualized as described above.

ation was assessed by measuring bromodeoxyuridine labeling using the Cell Proliferation ELISA (Roche).

Effects of Orlistat on Cell Death. Cells were plated in 96-well tissue culture plates in complete medium. After 24 h, the cells were exposed to Orlistat for an additional 24 h. Apoptosis was measured with the Cell Death Detection ELISA (Roche), which was performed according to the manufacturer's protocol. As an independent assessment of apoptosis, the amount of cleaved poly(ADP-ribose) polymerase was measured in cells after treatment with Orlistat. Cells were cultured with the Orlistat (25 μM) or ethanol for 72 h, or with Staurosporine (1 μM) for 5 h. At each time point, total cell extracts were generated by addition of 1$\times$ SDS sample buffer. Samples were subjected to Western analysis using antibodies against the cleaved form of poly(ADP-ribose) polymerase (Cell Signaling). Western blotting was performed according to protocols established by the manufacturer of the anti-poly(ADP-ribose) polymerase antibody

PC-3 Xenograft Tumor Model. The effect of Orlistat on growth of PC-3 tumors in nude mice was assessed with a staged model. PC-3 cells (1×10^6) were injected into the flank of male athymic nude mice 4–5 weeks of age. Tumors were allowed to grow until they reached a size of ~100 mm³, at which time Orlistat administration was initiated. Orlistat was administered in 30 μl of vehicle containing 33% ethanol and 66% PEG 400. Animals received 240 mg/kg/day of Orlistat. Tumor size was measured with calipers twice weekly, and volume was calculated with the formula volume = $\pi/6 \times XY^2$ (15).

RESULTS AND DISCUSSION

An activity-based proteomics screen was used to identify serine hydrolases in PCa cells and to screen for their inhibitors. Serine hydrolases were revealed with an activity-based probe composed of a
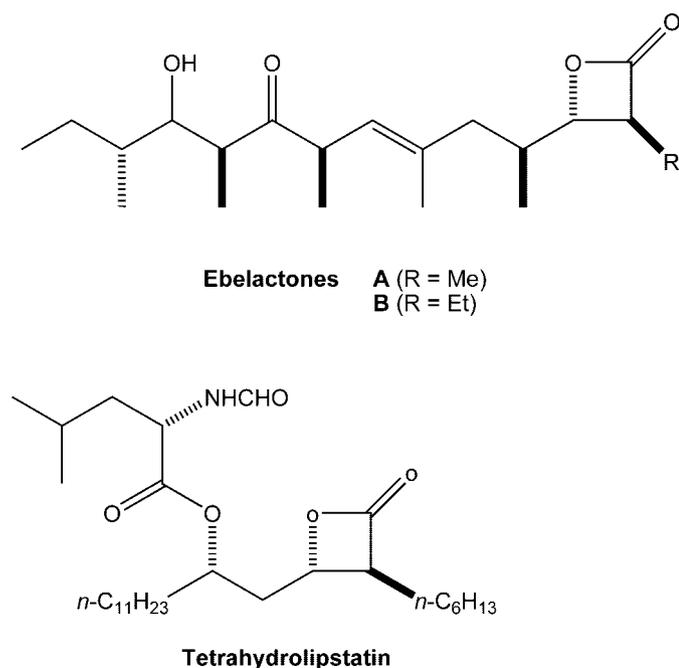

Ebelactones **A** (R = Me)
B (R = Et)

Tetrahydrolipstatin

Fig. 1. Structures of β lactones.

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FP warhead linked to the fluorophore (FP-TAMRA) (16). Primary cultures of normal prostate epithelial cells (PrEC) were compared with three PCa cell lines, LNCaP, DU-145, and PC-3. Cell lysates were reacted with FP-TAMRA and then resolved on SDS-PAGE (Fig. 2, *lanes 1–8*). In each case, ~15 different hydrolases were detected as fluorescent bands on SDS gels. The pattern of serine hydrolase expression is generally similar among the cell lines, with two significant distinctions. A band of 62 kDa was active in the normal PrECs but absent in all of the tumor lines. Peptide mass fingerprinting showed this enzyme to be carboxylesterase-2. Conversely, a hydrolase with a mass of ~270 kDa is expressed in all of the tumor lines but absent in normal PrECs. Peptide mass fingerprinting with mass spectrometry showed this band to be FAS, an observation that was confirmed by immunoprecipitating the complex between FP-TAMRA and FAS (Fig. 3B).

FAS is responsible for the conversion of dietary carbohydrate to fat and is the only eukaryotic enzyme capable of synthesizing palmitate, the precursor for the majority of nonessential fatty acids (17). FAS has a unique structure and mode of action. The enzyme contains seven separate enzymatic pockets and an acyl carrier protein. The distinct enzyme domains of FAS operate together to condense acetyl CoA and malonyl CoA, ultimately generating the 16 carbon polyunsaturated fatty acid palmitate. Palmitate remains covalently attached to the acyl carrier protein of the enzyme until it is liberated by the final enzymatic pocket on the enzyme, the intrinsic thioesterase. This thioesterase is the sole serine hydrolase within FAS and is the target of the FP-TAMRA probe (see below).

FAS is up-regulated in many tumors. Its function has been strongly linked to tumor cell proliferation (18), making it an attractive therapeutic target. A functional connection between FAS and tumor cell proliferation was originally suggested by work with the fungal product cerulenin and its synthetic derivative c75. These compounds inhibit the ketoacyl synthase domain of FAS, the first enzymatic pocket in the enzyme (19, 20), and have shown some promise as antitumor agents (8, 20–22).

We capitalized on the fact that FP-TAMRA reacts with the active site of the thioesterase domain of FAS to screen for alternative inhibitors of this enzyme. Three derivatives of natural products, each containing a β-lactone moiety, were tested for the ability to block activity-based labeling of FAS. These are ebelactones A and B, and tetrahydrolipstatin, which is also known as Orlistat and is marketed as Xenical (Fig. 1). The β-lactone can undergo nucleophilic attack on the carbonyl carbon of the lactone ring by the active site serine of the esterase, yielding a covalent adduct between enzyme and inhibitor (23). All three of the compounds inhibit the thioesterase of FAS (Fig. 2, *right panel, lanes 10–12*), but only Orlistat is selective for FAS in tumor cells. Orlistat is a drug approved and widely used for weight management in obese patients (24). The effectiveness of Orlistat in obesity is conferred by the ability of the drug to inhibit pancreatic lipase in the gastrointestinal tract, thereby preventing uptake of dietary fat. The inhibition of FAS by Orlistat has never been reported and is not believed to be relevant to its mode of action in weight loss.

To characterize the effects of Orlistat on FAS in intact cells, we measured the ability of the compound to inhibit the activity of FAS in whole cells. PC-3 cells were treated with a range of Orlistat, and the level of FAS thioesterase function was measured with a membrane-
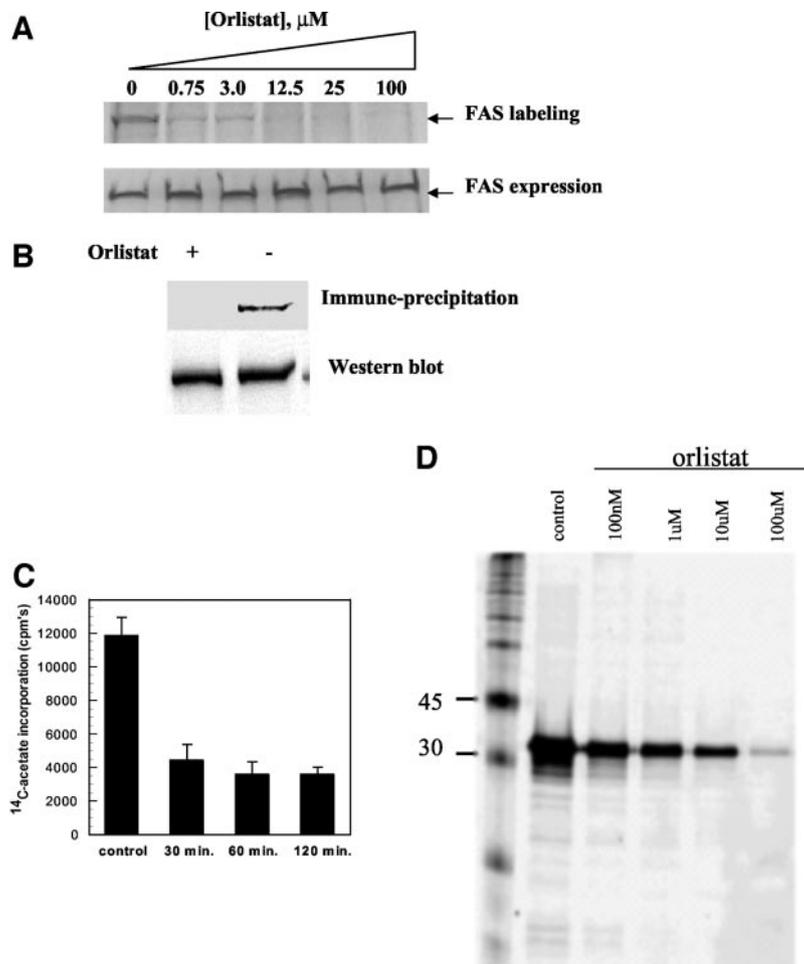

Fig. 3. Effect of Orlistat on cellular activity of fatty acid synthase (*FAS*). *A,* FAS activity in intact PC-3 cells was measured with a membrane-permeable activity probe, fluorophosphonate (FP)-BODIPY. PC-3 cells were first incubated with a range of Orlistat, and the remaining FAS activity was assessed by adding FP-BODIPY to the cells. Cell lysates were separated on SDS-PAGE, and active serine hydrolases were visualized (*top panel*) as described in "Materials and Methods." The effect of Orlistat on the level of FAS protein was measured using the same cell lysates as in by Western blot (*bottom panel*). *B,* immune precipitation of the complex between FAS and FP-6-carboxytetramethylrhodamine (TAMRA). Cell lysates were treated with 12 μM Orlistat (+) or vehicle control (−) before labeling with FP-TAMRA. FAS was immune precipitated with anti-FAS antibody. The *top panel* indicates the level of the complex between FP-TAMRA and FAS, whereas the *bottom panel* (*Western blot*) shows the amount of total FAS expression. *C,* the effect of Orlistat on cellular fatty acid synthesis was gauged by measuring the incorporation of [^{14}C]acetate into fatty acids as described in "Materials and Methods." *D,* the ability of Orlistat to block labeling of the recombinant thioesterase domain of FAS was measured by preincubating the thioesterase with a range of Orlistat and subsequently with the activity probe FP-TAMRA. Samples were run on SDG-PAGE, and signal was visualized as described; *bars,* ±SD.

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permeable activity-based probe, FP-BODIPY. Orlistat caused a concentration-dependent inhibition of labeling of FAS by FP-BODIPY (Fig. 3A), indicating that Orlistat inhibits FAS in intact cells. Orlistat had no effect on the abundance of FAS, which was measured from the same treated samples by Western blot. The identity of the labeled FAS was confirmed by immune-precipitating the complex between FP-TAMRA and FAS with an antibody specific for the enzyme (Fig. 3B). The effects of Orlistat on cellular fatty acid synthesis were gauged by measuring the incorporation of [^{14}C]acetate into fatty acids. Saturating levels of Orlistat (30 μM) reduced cellular fatty acid synthesis by ~75% within 30 min (Fig. 3C). Moreover, Orlistat blocked the labeling of the active site serine of the recombinant thioesterase domain of FAS by FP-PEG-TAMRA (Fig. 3D), proving that this enzymatic domain of FAS is a target for Orlistat. Because Orlistat is a tight-binding irreversible inhibitor, we cannot define its precise affinity for FAS. However, the results in Fig. 2D, and similar results obtained on whole FAS in cell lysates (not shown), suggest that the apparent K_i of Orlistat for FAS is near 100 nM. When treating whole cells, however, higher concentrations of the compound were necessary to achieve nearly complete inhibition of the enzyme (Fig. 3A). Orlistat has similar effects on FAS and on fatty acid synthesis in other PCa lines, as well as in colon and breast cancer cell lines (data not shown).

Orlistat induced a pronounced antiproliferative effect in the PC-3 cell line and exhibited a slight effect on the androgen-dependent LNCaP cells. The compound had little effect on the DU-145 cells or the normal PrEC cells in the 48-h time period of the measurement (Fig. 4A). The inhibitory effects of Orlistat on the proliferation of PC-3 cells were reversed by addition of palmitate, the end product of FAS (Fig. 4B). This observation strongly indicates that decreased proliferation results from inhibition of FAS by Orlistat.

We have observed that Orlistat has potent antiproliferative effects on many other tumor cell lines, including cells derived from breast (MDA-MB-435 and MDA-MB-231) and colon (Caco-2 and SW480) cancer. In virtually all of the cases, tumor cells are more sensitive to Orlistat over normal epithelial cells and fibroblasts. Yet, as observed here with PCa cells, variance in the sensitivity of tumor cells to Orlistat is observed (data not shown). The mechanisms underlying these differences are not entirely clear and are currently under investigation. The antitumor activity of anti-FAS compounds has been linked to hormone-dependence (25), PTEN status (26), and Her-2 status (27). Consequently, the sum total of these signaling pathways in any single tumor may ultimately define sensitivity to a FAS blockade.

Orlistat also induced tumor cell apoptosis, but again the sensitivity of the individual tumor cell lines to the compound was somewhat different (Fig. 5A). When cell death was assessed at 24 h by measuring DNA fragmentation (Roche Cell Death ELISA), the PC-3 and LNCaP cells exhibited substantial levels of cell death. At this time point, however, only a modest effect was observed on death of the DU-145 cells. Orlistat was without effect on death of human foreskin fibroblasts or normal PrECs. This finding is consistent with reports that FAS has little, if any, role in apoptosis in normal cells (18). Prolonged exposure (72 h) of each tumor cell line to Orlistat resulted in cleavage of poly(ADP-ribose) polymerase, another marker of apoptosis (Fig. 5B). In this and other apoptosis assays (e.g., staining of annexin on the cell surface), we have yet to observe significant effects of Orlistat (1–25 μM) on fibroblasts, normal PrECs, or normal mammary epithelial cells (data not shown). As with cell proliferation, the effects of Orlistat on apoptosis were also reversed by the addition of palmitate (Fig. 5C).

The effects of Orlistat on tumor growth in vivo were tested in a xenograft model with the PC-3 cells. Tumors were grown to ~100 mm^3 in the flank of nude mice, at which time Orlistat was adminis-
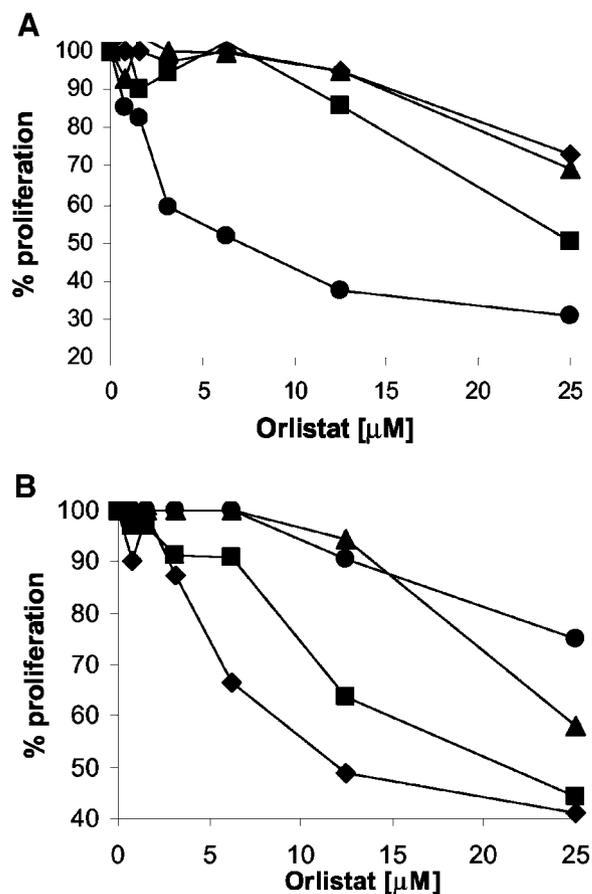

Fig. 4. Orlistat inhibits tumor cell proliferation. A, the effect of Orlistat on proliferation of prostate cancer cells was measured by the incorporation of bromodeoxyuridine (BrdUrd). PC-3 (●), LNCap (■), DU-145 (▲), and PrEC cells (◆) were seeded as subconfluent monolayers into microtiter plates. Cells were exposed to Orlistat for 32 h, then a BrdUrd labeling solution was added to the wells. BrdUrd incorporation was measured according to the manufacturer's protocol (Roche Cell Proliferation ELISA). Proliferation is expressed as a percentage of proliferation in untreated cells. B, the ability of palmitate to rescue cells from Orlistat-induced growth arrest was tested with PC-3 cells. Along with Orlistat, cells were incubated with 0 (◆), 3.25 μM (■), 7.5 μM (▲), and 15 μM (●) palmitate. BrdUrd incorporation was measured according to the manufacturer's protocol (Roche Cell Proliferation ELISA).

tered via i.p. injection (240 mg/kg/day). When compared with vehicle injection, Orlistat prevented the growth of PC-3 tumors (Fig. 6). In five separate experiments such as that shown in Fig. 6, tumor growth was blocked by 63%, 62%, 46%, 41%, and 16%. All of the differences were statistically significant in t tests with $Ps < 0.05$. Animals exhibited no outward signs of toxicity, experienced no loss of weight, nor were there any effects of Orlistat (240 mg/kg/day) on hematocrit or WBC levels (data not shown).

A pharmacokinetic analysis of Orlistat (155 mg/kg) administered by i.p. injection showed peak blood levels to be ~10 μM 2 h after dosing (data not shown). Beyond this time, blood levels of the drug decayed rapidly. Although the cost of a pharmacokinetic analysis at 240 mg/kg was prohibitive, it is unlikely that blood levels reached much beyond 16 μM, and the half-life is expected to be the same. Altogether then, the peak blood levels at the dose of 240 mg/kg (~16 μM) are likely to be just above the dose of Orlistat required to affect tumor cells (1–6 μM). These parameters are generally consistent with the level of growth inhibition that we observe in vivo.

Altogether, the findings presented here, and results from our larger survey of a number of tumor and normal cell lines (data not shown), lead to the following conclusions: (a) Orlistat is a novel inhibitor of the thioesterase activity of FAS, and by virtue of this property, Orlistat

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can block cellular fatty acid synthesis; (*b*) Orlistat can interfere with prostate tumor cell proliferation; (*c*) Orlistat selectively induces apoptosis in prostate tumor cells; (*d*) tumor cells have various levels of sensitivity to Orlistat; and (*e*) Orlistat inhibits growth of PC-3 xenograft tumors *in vivo*.

As with any compound, off-target activity is always a confounding issue. In fact, one reason why we chose to include Orlistat in our study was to determine whether Orlistat had targets other than pancreatic lipase. Nevertheless, the overwhelming body of evidence in this report indicates that the effects of Orlistat on tumor cells are elicited through its inhibition of FAS. Activity-based screening in numerous tumor cells has revealed no other target for Orlistat than FAS. Furthermore, the concentrations of Orlistat that elicit cytostatic and cytotoxic effects are very close to the cellular IC_{50} for the inhibition by Orlistat of FAS. Additional support for this conclusion is drawn from our finding
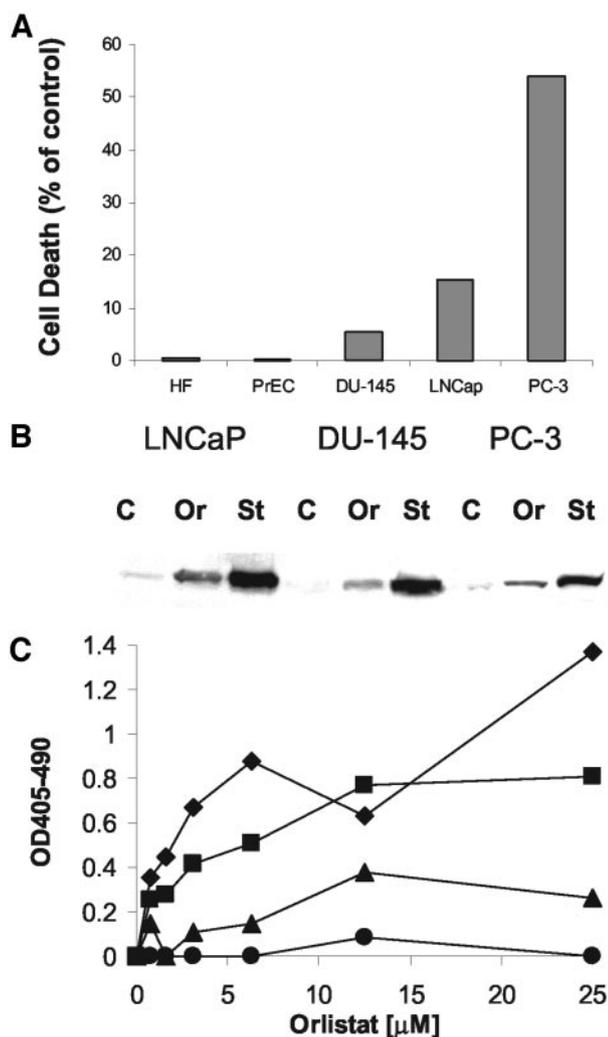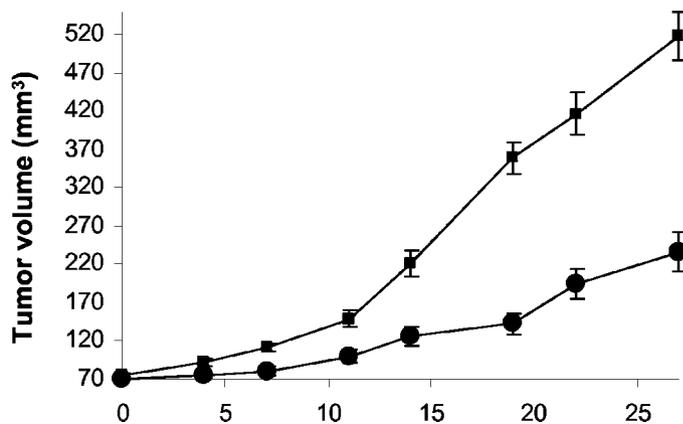

Fig. 6. Orlistat inhibits growth of PC-3 tumors *in vivo*. The effect of Orlistat on growth of PC-3 tumors in nude mice was assessed with a staged model. PC-3 cells (1×10^6) were injected into the flank of nude mice. Tumors were allowed to grow until they reached a size of ~100 mm³, at which time administration of Orlistat (●) or vehicle (■) was initiated in separate sets of 8 mice. Orlistat was administered at 240 mg/kg/day for a period of 3 weeks. This experiment is representative of four repetitions. The differences in mean tumor volume of the drug *versus* vehicle groups were statistically significant at every time point with the final measurement at day 28 having a *P* of 0.02 in a paired *t* test, assuming unequal variance; *bars*, ±SD.

that Orlistat directly inhibits the recombinant thioesterase domain of FAS. Finally, palmitate, the end product of FAS, rescues PC-3 cells from the antiproliferative and proapoptotic effects of the compound. This conclusion is also strongly supported by reports in the literature showing that FAS is misregulated in tumors (13) and that c75, an antagonist of the ketoacyl synthase domain, has antitumor activity (8, 20, 22). Finally, recent studies show that silencing of FAS in tumor cells with small interfering RNA induces apoptosis in PCa cells (28). Although we cannot exclude the possibility that Orlistat has some other target, the simplest interpretation of the data presented here is that Orlistat acts by inhibiting FAS.

Orlistat has minimal effects on the normal cells we have tested, suggesting that the compound could have therapeutic index sufficient for antitumor therapy. Orlistat also represents an alternative to cerulenin or c75, which inhibits the ketoacyl synthase domain of the enzyme but also interacts with carnitine palmitoyl transferase (29). In its approved formulation, however, Orlistat is administered orally. Because of its extremely low oral bioavailability, the effects of Orlistat are largely confined to the gastrointestinal tract, where it inactivates pancreatic lipase (24). Therefore, the formulation and route of delivery would have to be changed to treat tumors of the breast, prostate, and so on. One cannot exclude the possibility that the oral formulation of Orlistat could be useful in treating tumors of the gastrointestinal tract, such as colon cancer. We have found Orlistat to block FAS and induce apoptosis in a number of colon cancer lines,[3] so treating patients at high risk for colon cancer in a prophylactic manner could be considered.

The potential for synthesizing more potent or bioavailable variants of Orlistat is high. Orlistat is one of a class of compounds containing a reactive β-lactone. Other compounds in this class include the natural products ebelactones A and B, some inhibitors of HMG CoA synthase (30), and panclicin D, a synthetic inhibitor of pancreatic lipase (31). Given the relatively broad inhibition profile of ebelactones A and B against serine hydrolases (Fig. 2) and the demonstration that synthetic routes are available for creation of variants of Orlistat (30), a more in-depth evaluation of β-lactones as serine hydrolase antagonists and as antitumor agents is warranted.

Fig. 5. Orlistat induces tumor cell death. *A*, PC-3, LNCaP, DU-145, normal prostate epithelial cells (*PrECs*), and normal foreskin fibroblasts cells were exposed to Orlistat (12.5 μM) for 48 h. Tumor cell death was measured with the Cell Death Detection ELISA (Roche), which measures DNA fragments within immunocaptured nucleosomes. DNA fragmentation was assessed by measuring A_{405}-A_{490}. The level of cell death is plotted as a percentage of staurosporine induced death. *B*, LNCaP, DU-145, and PC-3 cells were cultured with Orlistat (12.5 μM) for 72 h, with Staurosporine (*St*) as a positive control for induction of cell death, or in medium without any stimulus (*C*). After incubation, cell extracts were generated and subjected to Western analysis using antibodies selective for the cleaved form of poly(ADP-ribose) polymerase. *C*, PC-3 cells were treated across a concentration range of Orlistat. Concurrently, cells were supplemented with palmitate. Control cells (●) received no palmitate. Test cells were treated with 1.8 μM (▲), 3.75 μM (■), or 7.5 μM (◆) palmitate. After a 48-h treatment, cell death was measured with the Cell Death Detection ELISA (Roche).

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[3] F. Axelrod. Orlistat as an Anti-Tumor Agent for Colorectal Cancer, manuscript in preparation.

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Finally, the identification of the ability of Orlistat to inhibit the thioesterase domain of FAS was made possible by the application of activity-based profiling. The analysis we performed involved a screen of only three β-lactones against slightly >35 different serine hydrolases. Even within this small test set, a novel target and indication were identified for an approved drug. It is reasonable to believe that a high throughput version of such a screen could drive decision-making in drug development. Information from such screens could lead to the identification of more selective leads much earlier in development. As in the case of this report, such an analysis might also point toward unanticipated targets and indications for other drugs.

ACKNOWLEDGMENTS

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